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EFFECT OF HUMAN DIABETIC PLASMA UPON BLOOD SUGAR CURVES IN RABBITS FOLLOWING INSULIN[1]

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THE OBSERVATIONS of Houssay and collaborators (1), of Long and Lukens (2) and of many others have left no doubt as to the profound influence of the pituitary and the suprarenal glands upon experimental diabetes and the metabolism of carbohydrate, protein and fat. It is one thing, however, to demonstrate this influence in depancreatized animals and quite another to prove that the same forces are operative in diabetes mellitus in man. Many facts strongly suggest that the findings in experimental animals may have a direct application to human diabetes, but conclusive proof of the existence of a truly 'diabetogenic hormone' is lacking. That prior to the onset of their disease persons who acquire diabetes in adult life are usually overweight and that in hyperpituitarism the incidence of glycosuria and diabetes is high, are facts which suggest that dysfunctioning of the pituitary gland may be connected with the existence of the diabetic state, but direct evidence to support this has not been brought forward.

The achievement of Young (3) in producing permanent diabetes in normal dogs by the intraperitoneal injection for 2 to 3 weeks of crude extracts of the anterior lobe of the hypophysis has more than ever directed attention to the possible rôle of this gland in human diabetes. Young's extracts may be characterized as diabetogenic in contrast to those which merely cause transient hyperglycemia and glycosuria, usually as an immediate response. Young's work has been confirmed by Campbell and Best (4) and by Dohan and Lukens (5). It was found that the removal of the pancreas from the experimental animal did not materially alter the severity of the diabetic condition; examination of the excised pancreas showed a marked decrease or absence of insulin (4) and extensive degeneration of the islet tissue (6).

During the past few years we have attempted to demonstrate in the blood or urine of diabetic patients some factor of a 'diabetogenic,' or even simply of a contrainsular, nature. In 1934 and 1935 we tried without success to show by the injection intravenously of blood serum or urine from diabetic patients an alteration in the blood

[1] This investigation was aided by a grant from the Proctor Fund of Harvard University.
[2] Elliott P. Joslin, M.D., Medical Director.

sugar curve following the giving of glucose intravenously to rabbits. This lack of success held whether the material from the diabetic patient was injected into the rabbit only once or for as many as three times on successive days prior to the test day. Because of this experience we were greatly interested in the report of de Wesselow and Griffiths (7) that the blood plasma of some elderly diabetic patients, especially obese females, when injected into rabbits, diminished the hypoglycemic action of insulin. Because of the importance of these findings we have tried to confirm them. We realized that blood plasma is a complex fluid of varied content and origin and that positive results would demand further work in order to identify the substance or substances in plasma responsible for effects observed. However, it seemed justifiable in preliminary studies to use whole, untreated plasma as did the English investigators.

METHODS

The procedure used was nearly as possible that of de Wesselow and Griffiths except for minor variations which seemed desirable to us. Rabbits 5 to 6 weeks old were taken for the tests, using a fresh animal for each plasma. The animals were chiefly of white Australian breed although a few were Dutch and hybrid Havana and Dutch. They were maintained on a diet of Park and Pollard rabbit pellets.[3] Food was withheld for 18 hours prior to the giving of the test dose of insulin. On the test day, 3 hours before the injection of insulin, 6 to 13 cc. of plasma from a diabetic patient was administered subcutaneously to the rabbit (in 20 of the 30 tests 10 cc. or more, and in 26 of the 30 tests 9.0 cc. or more, of plasma were used). The plasma was obtained from heparinized blood drawn under sterile conditions. In most instances the blood was obtained in the morning before the patient had had insulin or breakfast but in 7 instances it was taken during the course of an ordinary day. The time of day and the relationship to food appeared to have no influence on the results secured. No patient was used who had received protamine zinc insulin less than 24 hours previously. Three hours after the plasma had been injected into the rabbit, the animal was given intravenously 0.3 U of market (amorphous) insulin per kg. of body weight (Eli Lilly & Co. U_{10} strength diluted 10 times with sterile distilled water). The blood sugar of the rabbit was determined on 0.1 cc. samples obtained from an ear vein before and at 5 to 15 minute intervals up to 90 minutes after the giving of the insulin. The method of Folin and Malmros (8) was used for the analyses.

Control curves were obtained by carrying out the procedure as outlined above except that no plasma was injected. Two control experiments were performed with each animal, one about one week before and another one week after the day of the actual experiment, so that any effect of the variation in weight of the animals (unavoidable in young, rapidly growing rabbits) from week to week could be detected. Actually, the average weight of the rabbits used was 1275 gm. for the first control curve, 1468 gm. for the test curve one week later and 1597 gm. for the second control curve one week after the test curve. Inspection of figure 1 shows any effect of the variation in weight to have been negligible.

RESULTS

Since de Wesselow and Griffiths obtained the highest percentage of positive results, i.e., diminution in the hypoglycemic response to insulin, when plasma from elderly obese diabetics was employed, we proceeded at once to the use of plasma from

[3] Stated by the manufacturers to contain dried buttermilk, cod liver oil, rolled oats, alfalfa meal, O. P. linseed oil meal, corn meal, ground wheat, ground barley, oats, corn gluten meal, wheat bran, wheat middlings, bone meal, calcium carbonate, calcium phosphate, iodized salt and molasses. The analysis is said to be: protein at least 12%, fat at least 3%, carbohydrate at least 63% and crude fiber not over 12%.

such patients. In this group in the English series were 15 females and 1 male; accordingly we chose predominantly females.

In all, in our own tests, plasmas from 30 patients were used; of these patients, 8 were males and 22 females. The details as to age, height, body weight, duration and severity of the diabetes, and treatment are given in table 1. In figure 1 are composite

TABLE 1. DATA REGARDING PLASMA DONORS

No. and sex	Case No.	Age	Net height	Net weight	% of of normal weight	Duration of diabetes	Diet, gm. C	P	F	Daily insulin dose, u Unmod.	Prot. zinc	Sugar in test blood	Plasma injected
		yr.	cm.	kg.		yr.						%	cc.
1F	15098	65.0	164	56.7	88	3.3	141	71	100	24	104	0.15	11
2M	6516	20.9	185	81.6	112	9.9	170	90	91	16	24	0.15	10
3M	16247	54.3	183	95.3	114	5.3	163	84	97	0	36	0.12	11
4F	16284	62.7	167	59.9	88	0.8	134	71	93	20	32	0.22	13
5F	16409	59.5		84.4		14.1	134	70	96	0	24	0.19	9
6F	16408	63+	146	52.6	92	2.1	137	65	83	0	12	0.13	10
7F	5020	77.8	163	64.7	101	15.1	131	59	81	0	12	0.21	10
8F	14707	69.8	165	83.0	123	9.8	131	59	81	16	0	0.15	8
9M	16495	51.8	170	79.4	110	6.0	164	92	101	0	0	0.12	9.5
10F	14483	38.0	163	52.6	87	3.0	164	84	136	150	220	0.09	12
11M	16715	60.3	170	72.6	106	6.9	150	85	105	6	24	0.15	11
12F	13022	68.6	165	59.4	90	4.5	130	60	80	0	18	0.19	10
13F	7210	76.2	162	54.4	85	10.0	140	62	79	54	0	0.33	11.5
14F	16999	42.5	157	67.9	118	4.0	110	56	89	0	0	0.14	12.5
15F	12186	68.0	156	70.8	115	11.5	142	60	78	0	12	0.10	12.5
16F	17114	66.0	157	81.6	130	5.0	122	35	50	20	38	0.20	8
17M	5155	59.0	175	84.4	122	18.7	151	73	78	0	20	0.11	7
18F	12972	51.7	156	87.6	142	9.7	130	70	63	0	16	0.16	9
19F	17163	56.8	160	54.9	88	8.2	140	65	100	6	30	0.13	10
20F	17222	54.3	163	80.7	126	1.3	140	61	81	0	0	0.15	9
21F	17231	77.2	155	65.3	109	0.1	135	61	66	0	0	0.16	9
22F	16380	66.0	162	60.3	96	2.9	150	82	110	0	0	0.17	6
23F	17571	66.0	162	70.3	110	0.6	150	80	150	0	0	0.11	12
24M	17269	30.8	171	46.7	71	7.8	164	103	142	0	300	0.27	12
25F	17335	63.6	161	80.3	119	1.8	m. c. r.[1]			0	0	0.14	10
26F	17524	64.9	161	69.9	109	2.0	Free diet			0	0	0.32	9
27F	14555	52.8	166	73.5	106	7.1	130	76	66	10	20	0.30	12
28M	17162	34.0	178	68.0	95	0.7	123	49	54	960	400	0.23	12
29F[2]	15761	42.8	163	44.9	72	4.4	172	72	70	600	700	0.26	11
												0.14	10
												0.16	11
30M	18425	39.4	170	59	80	2.2	179	112	144	250	325	0.17	10

[1] Moderate carbohydrate restriction.
[2] Tests made following 3 daily injections of 11, 10 and 11 cc. of plasma.

curves showing the average values obtained in all control and all test experiments except for those with *cases 28* and *29*. No significant difference between the test and the control curves is evident.

In the further analysis of the data, three other charts similar to figure 1 were prepared to demonstrate differences, if any, which might be disclosed by special study of certain patients included among the 30 plasma donors, grouped as follows.

1. Eight patients with mild diabetes who were receiving no insulin; 7 of these were women.

2. Twelve patients whose body weight was 110% or more of the normal allowed by standard tables.

3. Five patients from group 2, who were receiving no insulin.

These charts are not reproduced here since, as in figure 1, the character of the blood sugar curve following insulin was not significantly altered by the previous injection of plasma.

The group of patients included 5 (*cases 10, 24, 28, 29, 30*) with an unexplained high requirement for insulin. Two of these deserve especial comment, because, of the 30 plasma donors used, these 2 were the only ones the injection of whose plasma appeared to alter the blood sugar response in rabbits after the standard dose of insulin. Brief summaries of their case histories follow.

Case 28 (D.M. 17162). Father J. P., born April 7, 1905, was first seen Sept. 19, 1938. Sugar had been found in his urine for the first time 5 days before and from symptoms the date of onset of his diabetic condition was estimated as June 1938. He spent from Sept. 19 to 28 at the New England Deaconess Hospital and was discharged with the following instructions: diet $C_{165}P_{90}F_{105}$; insulin, 6 U of crystalline plus 20 U of protamine zinc insulin daily before breakfast. He was readmitted in severe diabetic coma, critically ill, on Dec. 31, 1938 and required for treatment 510 U of insulin in the 9 hours after admission. At discharge from the hospital on Jan. 11, the diet was set at $C_{165}P_{100}F_{120}$ and the insulin dosage at 48 U of crystalline plus 64 U of protamine zinc insulin daily before breakfast. The patient was admitted for the third time on Jan. 17 in marked acidosis for which during the first 24 hours in the hospital he was given 980 U of insulin. On Jan. 26 while still in the hospital he again developed severe acidosis despite the fact that during the days just preceding, his insulin dosage had ranged from 300 to 480 U daily. From 8:15 A.M. on Jan. 26 to 6:45 A.M. Jan. 27,

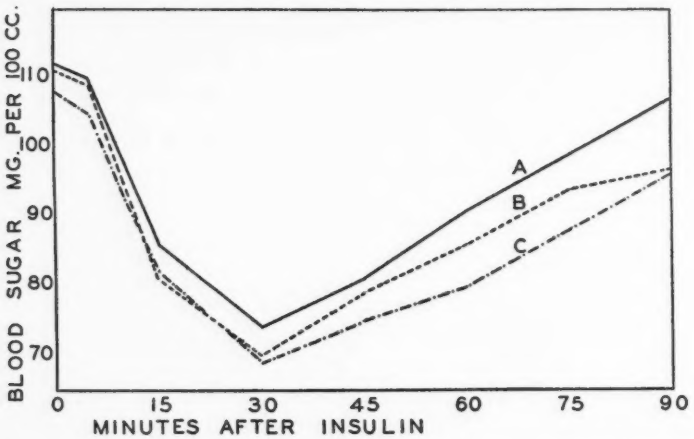

Fig. 1. COMPOSITE BLOOD SUGAR CURVES SHOWING EFFECT OF A SINGLE SUBCUTANEOUS INJECTION OF BLOOD PLASMA (FROM 28 DIABETIC DONORS) UPON THE RESPONSE TO INSULIN OF FASTING RABBITS. A. Control, one week before plasma injection. B. Test curve, three hours after plasma injection. C. Control, one week after plasma injection.

2312 U of insulin were given. The patient remained in the hospital until Feb. 24; during this period his daily insulin dosage varied from 520 to 1750 U. Following this his insulin requirement gradually and slowly fell until in September 1939 his condition was faily well controlled with 100 U of protamine zinc insulin daily.

Despite extensive studies, no positive clue as to the reason for the remarkable insulin requirement has been found. The data shown in figure 2 were obtained with blood plasma obtained on Feb. 3, 1939 while the patient was in the hospital and the diabetic condition was under fairly good control.

Case 29 (D.M. 15761), Miss C. L., born Aug. 14, 1896, was first seen June 13, 1937. Sugar had been found in her urine for the first time in June 1935 at which time treatment with diet and insulin had been instituted; from symptoms the date of onset of her diabetic condition was estimated as January 1935. She spent from June 13 to 19, 1937 at the New England Deaconess Hospital and was discharged with the following instructions: diet, $C_{154}P_{84}F_{100}$; insulin, 20 U of protamine zinc insulin daily before breakfast. She was readmitted on Jan. 2, 1939 with the history of an increasing requirement for insulin. She remained in the hospital until Jan. 18 at which time she was discharged taking 20 U of unmodified plus 44 U of protamine zinc insulin daily before breakfast. She was admitted for the third time on May 8, 1939 stating that she had been feeling well up until about April 1, although she had

been forced to increase her dosage of insulin gradually to 48 U of unmodified plus 60 U of protamine zinc insulin daily. About April 1 tests showed rather abruptly a marked increase in glycosuria. The patient had gradually increased the amount of insulin until by the time of admission to the hospital on May 8 she was taking approximately 500 U a day. In the hospital it was found that this was a real requirement. Consequently the amount was gradually increased until on June 17, 2120 U were given on one day. With the exception of 3 days when the amounts were slightly less, from June 6 to 22, 1800 U or more were administered daily. In the latter part of June and during July the insulin requirement gradually fell. At discharge from the hospital on Aug. 2, 1939, the dosage varied between 500 and 600 U daily; the patient was receiving a diet of the following composition: $C_{200}P_{86}F_{103}$. At the present writing (December 1939) the requirement has fallen to 350–400 U a day. As in the other patient just cited, studies have shown no definite cause for the remarkable insensitive-

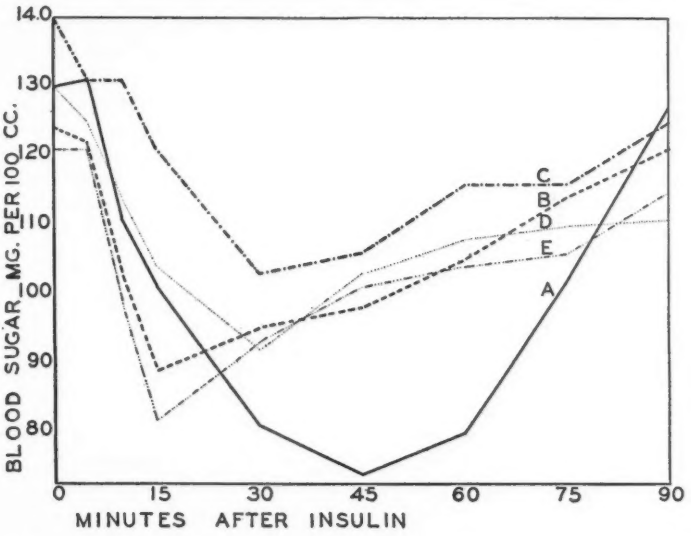

Fig. 2. Effect of a single subcutaneous injection of blood plasma from case 28 upon the response to insulin in a rabbit. A. Control, January 27, one week before plasma injection. B. Test curve, February 3, three hours after plasma injection. C. February 10. D. February 15. E. March 1. To avoid confusion, curves obtained on February 21 and March 17 are not shown. Plasma was given only in the experiment of February 3.

ness to insulin. The data shown in figure 3 were secured with blood plasma obtained on May 17, 1939, a day on which the urine was sugar free throughout and periods of hypoglycemia present; 1000 U of insulin were given on this day.

In figure 2 are shown the blood sugar curves obtained with the rabbit which received plasma from *case 28*. The control curve *A* obtained 1 week before the injection of plasma represented the usual response to the insulin given. The test curve *B* was obtained 3 hours after the subcutaneous injection of 12 cc. of plasma. The hypoglycemic effect of the insulin was significantly less than in the control experiment, suggesting that the plasma exerted a contra-insular effect. More surprising than this, however, was the control curve *C* obtained one week after curve *B*. In keeping with other results, we expected *C* to approach *A* in type; quite the contrary was the case. Because of this, other control tests were carried out with this rabbit at weekly

intervals for a total of 4 weeks after the injection of the plasma. The 4 other curves (2 of them have been omitted from figure 2 to avoid confusion) exhibited a gradual return to the type seen in the control curve *A*.

Somewhat similar results were obtained when *case 29* was used as the plasma donor. The results are shown in figure 3. The control curve *A* represents the customary response of the rabbit to the dose of insulin injected. The test curve *B* was obtained about 18 hours following the last of 3 consecutive, daily, subcutaneous injections of plasma in amounts of 11, 10 and 11 cc., respectively. Curve C was obtained 2 weeks after the test day; the effect obtained was much like that seen in curve C of figure 2. A response approaching that of the control curve *A* was obtained one week later or 3 weeks after the test day.

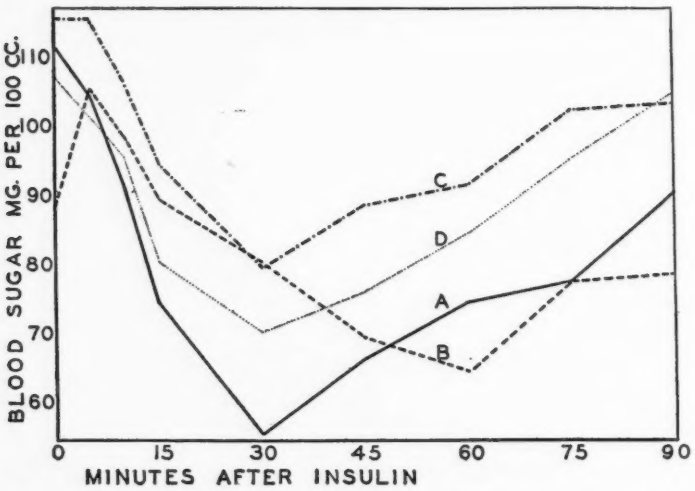

Fig. 3. Effect of 3 daily subcutaneous injections of blood plasma from case 29 upon the response to insulin in a rabbit. A. Control, May 9, before injection of plasma. B. Test curve, May 17, 18 hours after the last of 3 daily injections of plasma. C. June 1. D. June 8. Plasma was given only in the 3 days prior to May 17.

Attempts to confirm these results subsequently using other rabbits were unsuccessful; 4 additional animals were used with *case 28* and one other with *case 29*. The reason for this failure is not clear unless it represented an actual decrease in the "anti-insulin" effect of the plasma. It seems unlikely that the results secured were due to individual variations on the part of the rabbits used. It is true that the insulin dosage and degree of control of the diabetic condition of the two patients varied somewhat from time to time and that this might have influenced the results, but the differences as regards these features were not great. It is interesting that at a time when we were no longer able to demonstrate an 'anti-insulin' effect in rabbits, suggestive results were obtained in mice. These tests were carried out through the courtesy of Prof. C. H. Best of the University of Toronto. In a small group of mice the injection of serum from *case 28* appeared to confer some protection against hypoglycemic convulsions as compared to control animals. The series was too small to permit definite conclusions, however.

DISCUSSION

Our findings do not wholly confirm those of de Wesselow and Griffiths. No significant change was effected in the hypoglycemic response to insulin by the previous injection of the plasma of diabetic patients except when the plasma of 2 with high insulin requirements was used; these 2 will be discussed later. So far we have been unable to explain the discrepancy. As previously mentioned, minor differences in technique were introduced, but none of these seems adequate to explain the variation in results. Our rabbits were maintained on a diet different from that used by de Wesselow and Griffiths but like theirs it was high in carbohydrate. The English investigators used 0.2 U of insulin for all animals; our dosage was larger, 0.3 U per kg. of body weight of the rabbit, but in our hands this was necessary since the dose recommended by de Wesselow and Griffiths did not lower the blood sugar sufficiently to produce satisfactory control curves. Our control curves with the larger dosage are essentially of the same type as those of de Wesselow and Griffiths with the smaller dosage. The English workers do not describe in their paper the composition of the diet of their patients from whom blood was taken; our patients received from 130 to 170 gm. or more of carbohydrate daily (table 1).

Our series includes one patient (*case 11*) with acromegalic features and characteristic x-ray findings, although there was at the time no sign of hyperactivity of the pituitary gland. His plasma exerted no anti-insulin effect in contrast to the findings of Elmer, Giedosz and Scheps (9) who with one case of acromegaly obtained results similar to those of de Wesselow and Griffiths with their elderly obese diabetic patients.

Similar lack of confirmation of the results of de Wesselow and Griffiths has been reported by Dohan (10). On the other hand, Wilder (11) states that Rushton at the Mayo Clinic has obtained an insulin-inhibiting action in rabbits with the blood plasma of certain diabetic patients. Wilder mentions particularly a woman who developed insensitiveness to insulin during the same period in which symptoms of the menopause were present. Coincidental with treatment with large doses of estrin, the requirement for insulin decreased and the blood plasma no longer exerted a contra-insular effect.

It is possible that our results might have agreed more closely with those of de Wesselow and Griffiths if we had been able to use as plasma donors only women with onset of diabetes at the menopause and who at the time of the test had an increase in the blood prolan together with definite menopausal symptoms. However, it is not easy to assemble a group of such patients and insofar as we can make out, the patients of de Wesselow and Griffiths were not selected especially with this in mind.

The results obtained using the plasma of our 2 patients, *cases 28* and *29*, exhibiting marked insensitiveness to insulin, deserve further comment. As stated previously, a definite lessening of the hypoglycemic response to insulin in rabbits was seen during one series of tests with each plasma. Striking was the fact that the most pronounced alteration of the blood sugar curve was seen not within a few hours of the injection of plasma but 1 or 2 weeks later and that the apparent antagonism to insulin had not disappeared until 3 to 4 weeks after the injection. This strongly suggests that the plasma did not directly antagonize the action of insulin but had its effect rather by producing changes in the body of the test animal. An effect possibly analogous to this was observed by Young (12) who found that prior to the development of diabetes in dogs as a result of the daily injection of fresh extracts of the anterior pituitary, there occurred a phase of insulin resistance; indeed, in rats and rabbits this phase only, and not pituitary diabetes, could be produced.

Findings somewhat similar to ours were obtained by Glen and Eaton (13) in their detailed studies of a patient who received from 900 to 1050 U of insulin daily for 27 days. Injecting into rabbits only 2 cc. of their patient's serum, they found a definite decrease in the hypoglycemic response to insulin. The antagonism to insulin was more marked 48 hours after, than at the time of, the injection of serum.

It is interesting that the plasma of our other 3 patients, *cases* 10, 24 and 30, requiring large dosages of insulin, did not exhibit any anti-insulin effect when injected into rabbits. Glassberg, Somogyi and Taussig (14) likewise found no alteration in the response to insulin of 2 rabbits when 10 cc. of blood serum from a patient with insulin resistance were added to insulin at the time of injection.

SUMMARY

The hypoglycemic response to insulin of young rabbits fasted 18 hours was unaffected by the subcutaneous injection 3 hours previously of approximately 10 cc. of heparinized blood plasma from diabetic patients, chiefly middle-aged or elderly persons. Exceptions were observed in only 2 of the 30 patients who were used as plasma donors. These patients were two of a group of 5 (included in the 30) who had an unexplained high requirement for insulin with dosages ranging from 300 to 1360 U a day at the time of the tests.

When plasmas from these 2 patients were used, a definite though inconstant diminution in the hypoglycemic response to insulin was observed. The effect obtained seemed to be in the nature of an induced insensitiveness to insulin rather than a direct antagonism, since it became more pronounced 1 or 2 weeks after the injection of the plasma and did not disappear until 3 to 4 weeks after the injection.

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REVERSE OR 'PARADOXICAL' BLOOD SUGAR RESPONSE TO INGESTED GLUCOSE

A CORRELATION OF LABORATORY AND CLINICAL FINDINGS ON FORTY PATIENTS[1]

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A GLUCOSE TOLERANCE TEST made on *Miss V.Z.* (*Case 1*), April 12, 1930, gave results diametrically opposed to the accepted normal, that is, a depression of the blood sugar below the initial or fasting level for the 3-hour period of the test.[2] Since then to August 1, 1939, 38 similar reactions, or 5.6% of a total of 698 glucose tolerance tests, have been studied at the Polyclinic, and 1, *Case 24*, at the Columbus Hospital (*Case 16663*),— a total of 40; 10 males and 30 females. The purpose of this communication is to present the laboratory and clinical findings on this series of patients.

METHODS AND STANDARDS

The glucose tolerance test, hereinafter designated as G.T.T., was conducted as follows. The patient presented himself after a full night's rest and without having had breakfast. After a 30-minute rest in the recumbent position, a basal metabolic rate determination was made, a specimen of urine, and a blood sample per vena puncture, were obtained and the patient given 100 gm. of glucose in water and lemon juice. Sufficient blood was taken at the fasting period to supply an average of 8 laboratory procedures per person. Blood samples were taken at ½, 1, 2, 3 and 4-hour intervals following the ingestion of glucose—a total of 6 samples. The patient was permitted to lounge or move about, however, food, fluids and smoking were withheld during the course of the examination. A specimen of urine was collected, whenever possible, at each blood sampling period.

The various chemical tests were performed as described by Gradwohl (1). Quantitative blood sugar determinations were made by the Myers method and qualitative tests for sugar in the urine by the Benedict method. For the sake of convenience, the various physical and chemical procedures, together with standards of normal variations, are cited with the presentation of data.

PRESENTATION OF DATA

Reverse Response to Ingested Glucose

The data for the series of 40 reverse glucose responses are tabulated in table 1. The average initial or fasting sugar level was 140 mg.%,[3] a level of 20 mg., or 17%, greater than the accepted upper limit of normal variation. The level 30 minutes following the ingestion of glucose was 17 mg. below that of the fasting level,—a drop of 12%. The level at the 1-hour period was reduced to 113, a depression of 27 mg.

[1] Read before the Northwest Pacific Society of Internal Medicine, Vancouver, B. C., September 2, 1939.

[2] The clinical history of this case was presented before the Northwest Pacific Society of Internal Medicine at the meeting in Vancouver, B. C., September, 1931.

[3] Hereinafter designated as mg.

TABLE 1. REVERSE OR 'PARADOXICAL' BLOOD SUGAR RESPONSE TO INGESTED GLUCOSE

No.	Date	Sex and age	Glucose tolerance test, time in hr.						Urine sugar
			F	½	1	2	3	4	
1	4-12-30	24♀	112	111	75	73	83	—	0
2	7-12-34	12♂	99	56	45	55	77	—	0
3	7-10-35	22♀	95	81	95	82	91	42	0
4	9-11-35	15♀	111	93	63	80	67	63	0
5	1-11-36	42♀	121	111	80	54	101	60	0
6	10-16-36	40♂	119	100	70	71	73	66	0
7	11- 4-36	46♀	128	121	114	99	77	77	0
8	11-10-36	24♀	200	185	132	156	104	97	0
9	11-11-36	20♂	132	122	125	83	111	88	0
10	12- 2-36	27♀	161	100	128	119	130	67	0
11	12- 9-36	16♀	133	100	100	103	97	94	0
12	12-19-36	20♀	122	116	122	115	79	79	0
13	2-19-37	23♀	143	130	137	119	78	86	0
14	3-10-37	31♀	135	116	114	101	91	100	0
15	4-23-37	33♂	164	161	156	133	94	93	0
16	7-13-37	37♀	159	154	152	156	132	83	0
17	8- 2-37	62♀	222	213	185	156	145	64	0
18	9-14-37	28♀	156	153	111	141	128	118	0
19	10-16-37	37♀	156	128	132	118	95	112	0
20	11-17-37	26♀	143	105	92	121	118	118	0
21	11-18-37	23♂	133	86	118	93	78	95	0
22	11-30-37	34♀	161	152	152	98	93	95	0
23	12- 7-37	40♀	167	145	122	128	104	97	0
24	12- 8-37	28♂	100	80	70	66	90	100	0
25	1-18-38	24♀	156	141	133	118	70	79	0
26	1-19-38	9♂	145	139	111	137	125	116	0
27	1-26-38	15♀	162	118	113	139	143	130	0
28	1-27-38	64♂	175	152	162	133	128	137	0
29	4- 6-38	33♀	109	97	78	108	42	77	0
30	4-15-38	38♀	97	69	69	93	86	51	0
31	5-10-38	8♂	127	115	124	116	103	104	0
32	5-16-38	58♀	132	127	115	132	86	90	0
33	7- 1-38	28♀	145	139	114	89	89	98	0
34	7-22-38	17♂	156	143	116	110	135	91	0
35	7-25-38	29♀	135	114	100	121	79	77	0
36	9-20-38	7♀	135	132	128	96	98	81	0
37	9-28-38	14♀	141	130	95	104	112	83	0
38	4-24-39	17♀	167	133	112	105	141	107	0
39	5-19-39	42♀	122	116	113	120	142	119	0
40	6-16-39	45♀	137	133	128	105	106	102	0
Average			140	123	113	109	101	90	0
Maximum		64♂ 62♀	222	213	185	156	145	137	
Minimum		8♂ 7♀	95	56	45	54	42	42	
Variation		56♂ 55♀	127	157	140	102	103	95	

or 19% below the fasting level. The level at the 2-hour period was 109 mg. (22%); at the 3-hour period, 101 mg. (28%); and, at the close of the test, 90 mg., a total depression of 50 points or 36%.

The greatest fasting blood sugar reading was 222 mg. (*Case 17*), the lowest, 95 mg. (*Case 3*). Normal fasting levels, 80 to 120, occurred 8 times, and hyperglycemic levels, above 120, 32 times or 80% of the entire series. In 2 instances, (*Case 8, 17*), the sugar level was above that at which sugar usually is spilled by the kidneys, that is, above 180 mg. In neither instance, however, was sugar found in the urine, a condition described by Davidson as 'hyperglycemia without glycosuria' (2).

Sugar levels less than 80 occurred twice at the 30-minute period, 7 times at the 1-hour period, 5 at 2 hours, 10 at 3 hours, and 12 at the close of the test, a total of

36 or 18% of the 198 individual determinations made following the ingestion of glucose. Otherwise stated, 17 or 43% of the 40 patients developed hypoglycemic sugar levels (less than 80 mg.), 36 times following the ingestion of glucose, and of this number 11 patients developed readings less than 70 mg., 20 times or 28%.

On 7 patients of the present series, a total of 14 G.T.T. were performed subsequent to the initial one (table 2). In the series of subsequent tests the initial response to glucose was an average rise of 33 mg. or 30%. From this point, 144 mg., the level was reduced to 94 at the 4-hour period, a depression of 17 mg. (15%) below the fasting level and 50 mg. below the height of the initial rise. It is of interest to note that hypoglycemic levels, less than 70 mg., occurred 10 times in 5 patients in the initial and 8 times in 5 patients in the subsequent set of tests. Two only (*Case 2, 29*), of the 7 developed hypoglycemic levels during the initial and subsequent tests. Each of the 7 patients, however, developed hypoglycemic levels either in the initial or subsequent tests.

TABLE 2. RESULTS OF 14 SUBSEQUENT TESTS ON 7 PATIENTS SHOWING AN INITIAL REVERSE GLUCOSE REACTION

Pt. No.	Initial reaction							Subsequent reactions					
	F	½	1	2	3	4	Date	F	½	1	2	3	4
1	112	111	75	73	83	—	6-11-30	95	145	97	106	82	—
							6-18-30	101	167	137	101	65	56
							3-30-35	82	135	122	100	65	56
2	99	56	45	55	77	—	12-21-34	122	167	108	90	63	83
5	121	111	80	54	101	60	6-16-36	137	154	145	127	128	124
15	164	161	156	133	94	93	4-30-37	93	133	100	109	65	100
							6-16-37	91	127	125	119	83	80
							6-30-39	154	196	159	133	118	114
24	100	80	70	66	90	100	1- 4-38	101	105	115	120	124	106
							1-14-38	97	130	92	80	94	102
29	109	97	78	108	42	77	4-19-38	86	118	70	67	76	64
							1-10-39	133	154	105	110	100	114
							3-27-39	137	147	147	137	142	123
30	97	69	69	93	86	51	4-22-38	125	132	102	119	86	102
Ave.	114	98	82	83	82	76		111	144	116	108	92	94
Max.	164	161	156	133	101	100		154	196	159	137	128	124
Min.	97	56	45	54	42	51		82	105	70	67	63	56
Var.	67	105	111	79	59	49		72	91	89	70	65	68

Examination of the urine for the presence of sugar was made 195 times on the series of 40 patients, an average of 4.8 times per person. In no instance was sugar found either before, during, or after the G.T.T. Wassermann reactions obtained on 27 patients were entirely negative.

Measurable Findings Associated with Reverse Glucose Tolerance Tests

In addition to the 460 individual determinations on 3 procedures, 238 blood sugar, 195 urine and 27 Wassermann examinations, a total of 347 physical and chemical determinations were made on the series of 40 patients, that is, an average of 8 procedures per person of 11 procedures studied. Of this number of procedures 146, or 42%, gave results which lay outside the range of accepted normals (table 3, 4).

Physical measurements, weight. Deviations in weight greater than ±15% of the optimum (4) were arbitrarily assumed to represent abnormal variations. Variations greater than 15% of the optimum occurred in 17, or 45% of the patients: 9 overweight, and 8 underweight. On the other hand, if 20% variation from the optimum weight for sex, age and height is to be considered normal, then 8 of the series were over and 6 underweight, a total of 14, or 37%, abnormal variations. The

average weight for the series was 139 lb. or 6 lb. above the average optimum. The greatest individual increase was 129 lb., or 100% (*Case 25*), and the greatest decrease 43 lb., or 37%.

Red blood cell count (Male, 4.4 to 6.4; female, 4 to 5.6–5.8 million). Such counts were made on 10 males and 22 females, a total of 32. The greatest individual reading was 5,120,000 (*Case 32*, a female) and the lowest, 3,410,000 (*Case 28*, a male whose hemoglobin concentration was 13.5 gm.). Counts less than 4,400,000 occurred 5 times among the 10 males (50%), and counts of less than 4,000,000 were found on 5,

TABLE 3. LABORATORY FINDINGS ASSOCIATED WITH REVERSE GLUCOSE REACTIONS

No.	Height	Weight variation +	−	Pounds	B.M.R. +	−	Chol.	Salt	Ca	P	Ca:P	Urea N	Creat.	Wass.	Hematology Hb. male	Hb. gm. female	R.B.C.	W.B.C.
1	65.50	42	—	174	35	—	0	0	9.5	2.9	1:3.3	0	0	0	—	0	0	0
2	64.50	34	—	106	—	13	190	0	10.6	2.6	1:4.1	0	0	—	0	—	0	0
3	65.75	82	—	209	2	—	266	0	10.3	3.2	1:3.2	0	0	—	—	0	0	0
4	63.50	—	43	73	—	24	265	0	10.3	3.0	1:3.4	0	0	—	—	0	0	0
5	62.00	—	28	105	—	10	0	0	0	0	0	0	0	—	—	14.3	4,250,000	10,300
6	71.50	—	32	145	4	—	347	0	11.6	4.0	1:2.9	0	0	0	0	—	0	0
7	65.75	—	7	140	—	5	433	476	11.3	3.4	1:3.3	0	0	—	—	11.8	3,740,000	0
8	64.50	—	13	120	—	7	308	433	12.5	3.6	1:3.5	0	0	—	—	13.9	4,440,000	7,250
9	70.00	9	—	161	—	33	315	447	13.0	4.2	1:3.1	0	0	0	13.5	—	4,010,000	6,050
10	62.50	—	28	96	12	—	189	461	12.6	3.1	1:4.1	0	0	—	—	13.7	5,000,000	6,450
11	63.00	25	—	142	—	13	368	486	12.4	2.8	1:4.4	0	0	—	—	11.8	4,140,000	6,000
12	65.00	—	7	131	—	10	360	457	11.0	3.1	1:3.5	0	0	—	—	15.2	4,970,000	0
13	62.00	10	—	131	3	—	336	483	11.8	2.7	1:4.4	0	0	—	—	13.5	4,180,000	4,950
14	66.50	44	—	185	—	13	196	460	0	0	0	21.1	2.6	0	—	0	0	0
15	67.00	—	—	149	—	13	0	0	12.6	0	0	11.8	0	0	0	—	0	0
16	65.25	136	—	277	—	13	336	351	13.6	3.8	1:3.6	15.3	0	0	—	12.7	3,790,000	5,200
17	64.50	—	9	140	—	6	272	0	13.0	2.8	1:4.6	0	0	0	—	13.0	3,780,000	11,900
18	60.75	—	—	120	2	—	167	429	13.8	3.3	1:4.2	15.3	2.0	—	—	14.3	4,250,000	11,000
19	68.75	—	4	150	—	11	215	512	9.5	3.7	1:2.6	22.4	0.9	0	—	13.5	4,060,000	6,700
20	62.75	51	—	175	—	24	168	536	10.6	3.2	1:3.3	23.6	1.2	—	—	11.8	3,720,000	5,950
21	69.75	—	21	132	—	22	152	453	9.5	3.1	1:3.1	21.0	0.9	0	14.2	—	4,150,000	6,600
22	66.75	—	12	133	0	0	0	0	10.0	4.0	1:2.5	0	0	—	—	14.0	4,020,000	0
23	67.25	—	18	135	—	12	0	0	9.3	4.1	1:2.3	23.0	0	0	—	0	0	0
24	70.75	—	43	120	0	0	0	429	0	0	0	14.6	0	0	14.2	—	4,720,000	7,200
25	65.25	129	—	257	—	7	207	439	10.4	4.1	1:2.5	9.6	0.9	0	—	13.2	4,200,000	8,700
26	52.00	—	9	55	14	—	206	429	10.5	4.8	1:2.2	15.5	1.0	0	13.9	—	4,530,000	9,750
27	65.25	—	3	120	—	2	157	495	10.8	4.1	1:2.6	15.0	1.5	0	—	13.5	4,040,000	6,350
28	70.25	—	32	142	—	17	177	460	9.5	3.4	1:2.8	12.7	1.4	0	13.5	—	3,410,000	9,600
29	64.00	—	13	120	—	11	216	462	10.3	3.4	1:3.0	19.5	2.8	0	—	12.7	4,010,000	7,550
30	64.00	8	—	145	—	10	191	495	11.3	5.0	1:2.3	20.2	1.9	0	—	12.7	4,070,000	6,550
31	0	0	0	0	0	0	308	455	10.0	3.5	1:2.9	13.7	1.5	0	13.4	—	4,200,000	8,700
32	61.75	—	30	107	22	—	181	412	10.9	3.3	1:3.0	14.1	0.9	0	—	16.1	5,120,000	9,650
33	66.25	22	—	160	—	25	166	445	10.7	2.8	1:3.8	17.0	1.8	0	—	13.5	4,000,000	4,000
34	68.25	—	28	111	—	6	182	475	10.2	3.8	1:2.7	10.3	1.8	0	13.7	—	4,300,000	6,600
35	58.75	—	1	115	1	—	0	455	10.9	2.9	1:3.8	0	0	0	—	14.2	3,990,000	8,750
36	0	0	0	0	0	0	260	470	10.7	4.8	1:2.2	19.4	1.6	0	—	12.7	4,190,000	7,500
37	60.75	16	—	120	—	22	182	460	12.7	3.9	1:3.3	14.2	1.1	0	—	10.5	4,490,000	7,300
38	64.25	—	11	112	—	19	166	435	15.3	3.8	1:4.3	21.3	1.7	0	—	14.2	4,250,000	8,100
39	67.00	6	—	157	19	—	186	389	11.4	3.3	1:3.5	10.7	1.2	0	—	13.9	4,170,000	5,800
40	63.00	1	—	139	—	7	210	448	11.3	2.9	1:3.9	12.9	1.4	0	—	13.0	3,820,000	6,550
Ave.	64.90	41	19	139	11	14	239	455	11.2	3.5	1:3.3	16.4	1.5	0	13.8	13.4	4,187,000	7,482
Max.	71.50	136	43	277	35	33	433	536	15.3	5.0	1:4.6	23.6	2.8		14.2	16.1	5,120,000	11,900
Min.	60.75	1	1	55	1	2	152	351	9.3	2.6	1:2.2	9.6	0.9		13.4	10.5	3,410,000	4,000
Var.	10.75	135	42	222	34	31	281	185	6.0	2.4	2.4	14.0	1.9		0.8	5.6	1,710,000	7,900

or 23% of the 22 females. In other words, 10 of the 32 determinations, or 31%, had readings lower than the average normal.

White blood cell count (5,000 to 8–10,000). White cell counts were performed on 29 patients. The individual counts ranged from 11,900 (*Case 17*) to 4,000 (*Case 33*). In 10 instances, 34%, the counts were greater than 7,500. If the upper limit of normal is to be considered 10,000, then only 3 patients, or 10% had abnormally high readings.

Chemical measurements, basal metabolic rate (Sanborn: +10 to −10%). The B.M.R. was determined on 36, 90%, of the 40 patients. In 10 instances the readings averaged +11% and, of this number, 5 were greater than +10 (tables 4, 5). Twenty-six determinations, on the other hand, averaged −14%, and of this number 16 were less than −10%. The highest individual reading was +35% (*Case 1*), and the

TABLE 4. THE NUMBER AND VARIATIONS OF MEASURABLE FACTORS AND CLINICAL STATES ASSOCIATED WITH 40 REVERSE BLOOD SUGAR RESPONSES TO INGESTED GLUCOSE

Factors considered		No. tests	Variations above	Variations below	No.	%
A. Blood sugar (321 individual determinations)						
1. Glucose tolerance tests						
a. Initial series (238 readings on 40 patients)		40	—	40	40	100
b. Subsequent series (83 readings (14 G.T.T.) on 7 patients)		14	14	—	14	100
2. Blood sugar levels, 70 mg. or below						
a. Initial series		40	10	—	10	25
b. Subsequent series		7	5	—	5	71
B. Urine sugar (195 individual determinations)		40	—	40	40	100
C. Blood Wassermann		27	—	27	27	100
D. Physical variations	Normal variations					
1. Weight	+15 to −15%	38	9	8	17	45
2. Red blood cell count	M. 4.4 to 6.4 Mil.; F. 4 to 5.8	32	5	5	10	31
3. White blood cell count	5 to 10,000	29	11	1	12	41
Total		99	25	14	39	39
E. Chemical variations (blood)						
1. Oxygen consumption (B.M.R.)	+10 to −10%	36	5	16	21	58
2. Cholesterol	180 to 220 mg.	33	14	7	21	64
3. Sodium chloride	450 to 500 mg.	30	3	10	13	43
4. Calcium	9 to 11 mg.	37	16	0	16	43
5. Inorganic phosphorus	3 to 4 mg.	36	7	8	15	41
6. Urea nitrogen	10 to 15 mg.	24	13	1	14	58
7. Creatinine	1 to 2 mg.	20	2	4	6	30
8. Hemoglobin	M. 13 to 20 gm.; F. 11 to 16	32	0	1	1	3
		248	60	47	107	43
F. Clinical diagnoses		40				
1. Nervous and mental diseases					29	72
2. Diseases of the generative systems					9	23
3. Cardio-vascular-renal diseases					8	20
4. Focal and general infections					8	20
5. Allergy					7	18
6. Avitaminoses					6	15
7. Dermatoses					5	13
Total		40			72	183
Summary of abnormal variations						
1. Reverse response to glucose		40	40	—	40	100
2. Physical		99	25	14	39	39
3. Chemical		248	60	47	107	43
4. Clinical		40	72	—	72	183
Total		427	197	61	258	60

Number procedures employed	14.
Average number procedures per person	12.15
Average number abnormal findings per person	6.45

lowest −33, (*Case* 9). In other words, 21, or 58% of the 36 determinations were outside the range of normal variations.

Cholesterol (Bloor: 140–170 to 170–220 mg). Cholesterol determinations were made on 33 of the 40 patients. The average of these readings was 239 mg., a figure 19 points higher than the accepted normal of 220. Individual readings varied from 433 (*Case* 7), to 152 mg. (*Case* 21). In 14 instances the readings were greater than 220 and in 7, less than 180 mg., that is, 21 or 64% of the readings were outside the range of normal variation.

Chlorides (Whitehorn: 350–570 to 500–620 mg.). Determinations of the blood chlorides as sodium chloride were made on 30 patients. The individual readings varied from 351 (*Case 16*) to 536 mg. (*Case 20*). In 3 instances the readings were greater than 500 mg., and in 10 instances less than 450, a total of 13, or 43% variation from the normal.

Calcium (Clark: 9 to 11–14 mg.). The average of 37 calcium determinations was 11.2 mg. The range of variation among the individual readings was from 9.3 to 15.3. Readings above 11, or the upper limit of normal, occurred 16 times, 43%.[4]

Inorganic phosphorus (Benedict: 3 to 4 mg.). Thirty-six phosphorus determinations gave an average reading of 3.5 mg. The greatest individual reading was 5.0, the lowest 2.6. In 7 instances the readings were greater than 4, and in 8 less than 3 mg. If a variation of 3 to 4 mg. is accepted as the range of normal variation, then 15, 42%, of the 36 determinations lay on either side of the normal range.

Urea nitrogen (Folin and Wu: 7–12 to 15 mg.). This test was made on 24 patients, and gave an average result of 16.4 mg., a figure higher than the accepted normal of 15. The greatest individual reading was 23.6 mg. (*Case 20*), the lowest 9.6 mg. (*Case 25*). Thirteen of the readings were greater than 15 mg. and 1 less than 10 mg., a total of 14, or 58%, which lay outside the normal range.

Creatinine (Chiles: 1 to 1.6–2.5 mg.). Six, or 30% of the 20 creatinine determinations lay outside the range of normal variation: 2 above, and 4 below. The greatest individual reading was 2.8 and the lowest 0.9 mg. The average reading for the series was 1.5 mg.

Hemoglobin (Neucomber: male, 13.5–14 to 20 gm.; female, 11 to 16–16.5 gm.). Hemoglobin determinations were made on 32 patients and only 1, *Case 37*, a girl of 14 years, had a reading (10.5 gm.) less than the lower range of normal variation. In no instance was the hemoglobin concentration greater than the upper range of normal.

Each of the 40 patients, as already implied, had 2 measurable constants in common: 1, a reverse blood sugar response to ingested glucose; and 2, urine free from sugar. Determinations of 11 measurable factors, other than the Wassermann reaction, on the other hand, gave various degrees of abnormal findings associated with the 2 constants (table 5). For instance, 99 physical measurements resulted in a total of 39, or 39%, variations from the normal averages. Further, a total of 248 chemical procedures resulted in 107, or 43%, variations beyond normal limits. There were, therefore, 146 readings of the total number of 347 which lay beyond the limits of normal variation, that is, 42% of the 11 measurable procedures lay in either direction beyond the range of accepted normals. Stated in other terms, each person of the series upon whom measurable procedures were performed had an average of 3.65 abnormal readings in addition to a reverse G.T.T. response and periods of blood sugar levels less than 70 mg.

The results of measurable procedures have been presented in terms of variations from accepted normals rather than the number and percentage of deviations in either direction beyond those accepted as normal. This method was deliberately chosen for the reason that it appeared a more accurate procedure when attempting to correlate 2 or more factors, each of which was subject to variations in 2 directions. For example, leanness and obesity, it would seem, do not constitute 2 independent

[4] An unpublished study of 1,487 blood calcium determinations on private patients at the Polyclinic from January, 1931 to July 20, 1939, leads me to accept a range of variation of 9 to 12.9 mg. as the average for clinical cases in and about Seattle. Of the total number of readings, 1,235, 83%, fell in this range of variation. There occurred, on the other hand, 141, 9.5%, readings less than 9 mg. and 111, 7.5%, above 12.9. The individual readings varied from 5 to 22 mg.

biological entities but rather directional extremes in one factor, namely, weight. Variations in the factor, weight, are variations in degree and it is this degree that excites clinical interest. Similar arguments hold for information relative to the other factors herein presented. For instance, 8 chemical procedures were carried out 248 times on the series of 40 patients, and of this number, 107, or 43%, lay on either side of the normal. This represents a ratio of 2.31 normal findings to 1 abnormal. Deviations above the normal occurred 60 times, 56%, or a ratio of 1.78 to 1, and deviations below the normal appeared 47 times, a ratio of 2.28 to 1. The ratio of findings above the norms to those below was 1.28 to 1.

Clinical States Associated with Reverse Blood Sugar Responses to Glucose

A total of 72 clinical diagnoses, aside from those established by laboratory procedures, were made on the series of 40 patients, that is, an average of 1.8 per person. The actual number of positive diagnoses per person ranged from 1 to 4. The diagnoses per number of persons afflicted, referable to systems and general states, were as follows: nervous, emotional and mental diseases, 29, or 73% of the series of 40 patients; diseases referable to the generative organs, 9, or 23%; cardio-vascular-renal diseases, 8, or 20%; focal and general infections, 8, or 20%; allergy, 7, or 18%; avitaminoses, 6, or 15%; and dermatoses, 5, or 13%.

The 146 variations beyond normal physical and chemical measurements together with the 72 clinically established abnormal states, gives the rather startling total of 218 observed deviations in the series of 40 patients, each of whom was selected for this study because of an abnormal metabolic reaction, namely, a reverse blood sugar response incident to the ingestion of glucose. In other words, each individual of the series upon whom determinations were made showed an average of 6.45 demonstrable deviations from accepted normals, and of this number 5.45 were found as secondary deviations to the disturbance in glucose metabolism.

In brief, a summary of the present findings (table 4) show that 43% of 11 factors measurably determined on 40 patients, each characterized by a reverse response to glucose, lay outside the range of normal variation. Stated in per cent of the number of determinations made, the figures are as follows: deviation in cholesterol, 64%; urea nitrogen and oxygen consumption, or B.M.R., each 58; weight 45; sodium chloride and calcium, each 43; inorganic phosphorus 42; white cell count 34; red blood cell count 31; and creatinine 30%. The urine was sugar free and the Wassermann reaction negative (27 determinations) in 100% of the cases studied. On the other hand, each of the 40 patients averaged 1.8 clinically diagnosed abnormal conditions.

If the normals as stated are acceptable, these figures, although small in number, suggest a definite relationship between reverse blood sugar responses to ingested glucose and a widespread state of instability in other metabolic processes. Save for cholesterol, oxygen consumption, inorganic phosphorus and possibly weight, the percentage of deviations as observed in the present study is not such as to warrant the contention that they are concomitant with disturbances in glucose metabolism. On the other hand, deviations in weight, and the metabolism of cholesterol, inorganic phosphorus and oxygen are well established accompaniments of disturbed glucose metabolism and, to this degree, the present study adds confirmation.

DISCUSSION

The present series of patients portrays an array of abnormal deviations associated with a profound disturbance in glucose metabolism. This latter disturbance, on first thought is highly suggestive of the clinical syndrome, hyperinsulinism. Upon critical analysis, however, it appears questionable to offer this series of cases as representative

of such a condition even though the blood sugar variation, in each instance, was toward hypoglycemic levels. Hyperinsulinism, as I understand it, is characterized by the spontaneous occurrence of hypoglycemic states productive of symptoms ranging from uneasiness to acute mania, and accompanied by a multitudinous array of observable manifestations. Further, the condition is relieved, and not, as in the present observations, precipitated by the ingestion of glucose. Lastly, anatomical information as to the pancreas is entirely lacking in the present study, hence a diagnosis of hyperinsulinism would be a highly inferential one.

The high fasting blood sugar level, an average of 140 mg. for the series, suggests a state of mild hyperglycemia rather than one of hyperinsulinism. Each of the 40 patients, however, responded to the ingestion of glucose in a manner analogous to that following the administration of insulin, that is, by an initial fall in the blood sugar concentration. For instance, *Case 17*, an elderly woman, had a fasting blood sugar of 222 mg., a definitely diabetic level. Following the ingestion of glucose, the blood sugar level dropped 158 mg., (222–64), or 71%, throughout the 4-hour period of the G.T.T. Similarly, *Case 8*, a young woman who had a high fasting blood sugar level, namely 200 mg., developed a level of 97 mg. at the 4-hour period, a fall of 103 mg., or 51%. On the other hand, 9 patients (*Case 2, 4, 5, 6, 10, 24, 25, 29, 30*) who had an average fasting sugar concentration of 119 mg., developed hypoglycemic levels, 70 and below, 19 times during the G.T.T. In 2 instances (*Case 3, 29*), the sugar level was reduced to 42 mg. If *Case 17*, who had an initial level of 222 mg. and developed a level of 64 mg. at the 4-hour period, is included with the 9 just mentioned, then 10, or 25% of the series of patients developed hypoglycemic levels, or evidence of a state of hyperinsulinism, 20 times in addition to executing a reverse response to the ingestion of glucose.

At this point, attention is called to a series of 24 patients, the glucose tolerance response of which was characterized by an initial depression, 16 mg. at the half-hour period, followed by an elevation of the blood sugar level to an average height of 5 mg. above the fasting level. Following this elevation, the sugar levels fell during the remainder of the test to 105, a point 22 mg. below the fasting level, or 6 greater than the initial fall. Sugar was found in the urine of 2 patients of this series.

The two sets of observations are characterized by a common factor, namely, depression of the sugar level as the initial reaction to the ingestion of glucose. In the first set, the blood sugar lowering factors were sufficient to maintain the level below that of the fasting state for the duration of the test, that is, 4 hours. In the second series, however, the depressor factors were insufficient to maintain the level below that of the fasting one. These findings, therefore, indicate that the ingestion of glucose excited factors which progressively reduced the blood sugar concentration below that of the fasting state during the 4-hour period of the G.T.T. of the initial series and during the first 30 minutes of the second series.

The present findings, while suggestive of insulin reaction, may represent a reduced or unopposed action of insulin; that is, a reduction in the activity of factors normally functioning to prevent depression of the blood sugar level. For instance, the present findings may be indicative of a functional inadequacy of the adrenal glands. Such an assumption is supported by Peters and Van Slyke (7) who state that epinephrin is a direct antagonist of insulin "because it attacks the same point in the carbohydrate cycle, exactly reversing the effect of insulin." Further, Britton et al., (8) have shown that hypersusceptibility to the hypoglycemic effect of insulin occurs following removal of the suprarenal glands. Lastly, Crandall and Cherry (9), from their studies of the hepatic glucose output in adrenalectomized animals, concluded

that the adrenal secretion is stimulated when the blood sugar reaches levels of from 60 to 50 mg.[5]

The figures given in this paper relative to deviations from normal variations in certain quantitative chemical analyses are open to serious doubt as to their significance. Such doubts have been engendered because of the lack of unanimity among authorities as to what constitutes a 'range of normal variation.' For instance, the lowest point of normal variation, as given by 4 authorities on 4 procedures (1, 4, 5, 6), shows an average variation of 25% from the average of the 4 lower limits stated to be normal. That is, the figures given by the various authorities for the lower limit of normal show a variation of 20% for cholesterol, 42% for sodium chloride, 30% for urea nitrogen, and 4% for hemoglobin in the male. On the other hand, the figures given for the upper range of normal varied from 1 to 29%, or an average of 13% for 8 procedures.

The present results on cholesterol determinations are illustrative of the confusion due to the lack of a set of standard constants and variables. Cholesterol values were obtained on 33 patients, the average of which was 239 mg. As stated earlier, 14 of the readings were greater than 220 mg. and 7 less than 180 (6), or a total of 21, or 64%, of the series of determinations. The same set of cholesterol readings, if judged by another set of standards stated to represent normal variations (1, 4, 5), for instance 140 to 170 mg., would give results as follows: normal readings, 6 or 18%; no reading below 140 mg., and 27, or 82%, above 170 mg. That is, according to one set of standards, 82% of a given series of determinations represent a variation above the upper limit of normal, while with another set of standards, a total of 21 tests show deviation from normal in 2 directions, 7 less and 14 greater. Further, according to one standard, 7 patients had hypocholesteremia and 11 were normal, and according to another standard, 6 were normal and the others suffered from hypercholesteremia.

A similar uncertainty applies for the upper and lower range for sodium chloride readings, the lower limit for urea nitrogen and the upper limit for creatinine. Confusion of this nature makes the use of the prefixes hyper- and hypo- of very dubious value in presenting laboratory findings as indicative of chemical states, and of this I am acutely aware in relation to the present communication.

The clinically diagnosed disturbances, save those referable to the nervous system, numerically are of little significance in the present study. A percentage correlation between a constant and a variable of from 13 to 23, as in the present case, more than likely, should be considered in the category of casual findings. On the other hand, an associated disturbance of greater than 50% undoubtedly is of significance. Hence, a 73% association of nervous disorders with reverse responses to glucose, is of some importance.

In the present study, 38 diagnoses referable to the nervous systems were made on 29 of the 40 patients. Those diagnoses were as follows: neuroses and psychoses, 12 times; myasthenia or fatigue syndrome, 11; ophthalmic migraine 4; feeble-mindedness 3; idiopathic epilepsy 2; and one each of the following: encephalitis, progressive muscular dystrophy, diabetes insipidus, amnesia incident to arteriosclerosis of the brain, morphinism, and habitual criminalism.

A consideration of the present findings in no way warrants a contention that the

[5] Since presentation of this paper, Heilbrun and Liebert (10) have demonstrated a direct relationship between insulin-induced hypoglycemic states and the amount of adrenalin in the circulation,—"The curves of those patients evidencing shock showed an inadequate adrenal response to insulin 90 minutes after the injection, while the non-shock curves displayed a marked rise in the adrenalin level of the blood at this time."

clinical conditions diagnosed were causative factors in the production of reverse blood sugar responses to ingested glucose. On the other hand, it might be contended that certain neurological conditions were manifestations of disturbed glucose metabolism. So, also, it might be contended that certain of the neurological findings were incident to disturbances, as already shown, found associated with processes other than those pertaining to the metabolism of glucose.

SUMMARY

Laboratory and clinical findings are presented on 40 patients, each of which showed a reverse or 'paradoxical' blood sugar response to the ingestion of glucose, and a sugar free urine.

The average fasting blood sugar level for this series was 140 mg., or a figure indicative of mild hyperglycemia. Blood sugar levels of 70 mg. or less were found 20 times on 10 patients during the G.T.T. The initial blood sugar response of 14 G.T.T. performed on 7 patients subsequent to the initial tests was an average elevation of 33 mg. above the fasting level, followed by a maximum depression of 19 mg. below the fasting reading. Five patients in the series of 7 initial tests developed hypoglycemic sugar levels 10 times. A like number of patients developed hypoglycemic levels 8 times in the 14 subsequent series of tests. Two patients only, however, developed hypoglycemic levels in each of the 2 series.

Ninety-nine physical, and 248 chemical, a total of 347 determinations were made on the 40 patients in addition to those incident to the G.T.T., urine and Wassermann examinations. A total of 39, or 40% of the 99 physical determinations lay beyond the limits of normal variation. A total of 107, or 43% of 248 chemical determinations, lay outside the range of figures assumed to represent 'normal variations.'

Seventy-two clinical diagnoses, aside from those established by laboratory procedures, were made on the 40 patients. Diagnoses referable to disturbances of the nervous system were made on 29, or 73%, of the 40 patients.

A correlation of the various findings shows that each of the 40 individuals studied had an average of 5.5 deviations from normal standards, in addition to the primary one, or reverse response to the ingestion of glucose.

It may be concluded that a rather large number of physical and chemical abnormal variations and diagnosable clinical states are found associated in various degrees with reverse or 'paradoxical' blood sugar responses to the ingestion of glucose. It may be concluded also, that the medical management of patients showing reverse glucose responses, as demonstrated on 7 patients, results in the amelioration of the symptomatology and a return of the glucose response toward the usual normal.

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BIOASSAYS FOR URINARY ANDROGENS

A COMPARISON OF THE RESULTS IN NORMAL MEN WITH THOSE HAVING ENDOCRINE DISTURBANCES

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THIS PAPER is a report of assays for urinary androgens in normal men, and in patients showing evidence of endocrine disorders relating particularly to the pituitary gland and the testes. The method employed for extraction and assay is that described by Gallagher and Koch (1, 2), employing the 15-minute acid hydrolysis. Five capons were used in each assay and 5 controls were injected with androsterone. The method of calculation used is a modification of that used by Koch and coworkers (1) and is described by McCullagh and Cuyler (3). All assays reported here were made on 24-hour urine specimens.

In some instances, in normal men, we employed the method of dibutyl ether extraction described by McCullagh and McLin (4). Where this method was used, the assays are so designated. For practical purposes, we feel that both methods may be used without distinction because of the close comparison found in the yield of urinary androgens. Thirty-nine assays have been made on specimens from 20 normal men who ranged in age from 23 to 37 years. Usually the specimens were collected several weeks apart. In 16 of the assays in the normal group extraction was made by Koch's benzol method, using the 15-minute acid hydrolysis, while 23 were extracted by the dibutyl ether process. Table 1 shows the age, weight, urine volume and assays.

By plotting the number of international units of androgenic substance excreted per day against the body weight of the normal subjects and against the urine volume of each specimen, we have found a lack of any relationship in both instances.

There are some rather marked variations in each individual, the greatest being a difference of 54 I.U. in *case 8* (table 1). The single assays ranged from 16 to 86 I.U. An average level for all normals assayed by both methods is 37.8 I.U. per day.

Peterson, Gallagher and Koch (2) found that by hydrolyzing the acidified urine for only 15 minutes, an increase of about 66% in the yield of androgenic substances was obtained, as compared to that obtained by a 2-hour hydrolysis.

The urinary androgen excretion of normal men has been studied by Gallagher et al. (5) who assayed the urine of 4 normal men over a 6 weeks' period. The consecutive urine specimens varied markedly in androgen content. However, they found that the average daily excretion for the 4 men was remarkably constant, ranging from 38 to 41 I.U. per day, while variations in the daily excretion ranged from 13 to 79 U. This would correspond to 63 to 69 U for the daily average and 22 to 132 U for the daily range if calculated in equivalents of levels obtained by the 15-minute hydrolysis method. The ages of their subjects were 26, 27, 30 and 35 years.

Pituitary Syndromes, Pituitary and Parasellar Lesions

Under this title, 6 groups are presented: (*a*), proved pituitary tumors and parasellar lesions, 7 cases; (*b*), acromegaly, 5 cases; (*c*), Cushing's syndrome, 2 cases;

TABLE 1. TWENTY-FOUR-HOUR URINARY ANDROGEN ASSAYS ON NORMAL MEN

Case	Age	Weight	24-hour volume	Benzene extraction assay	Dibutyl extraction assay	Average
		lb.	cc.	I.U.	I.U.	I.U.
1	27	150	1690	25		
			1680		35	30
2	29	180	900	86		86
3	37	240	2950	42		
			—	43		
			2650		76	53.7
4	28	132	720	51		
			870		24	
			1030		35	32.7
			580		18	32.7
5	30	135	1300	21		21
6	33	170	800	32		
			680	16		
			690		22	
			1450		22	23
7	33	145	950	46		
			1900		54	50
8	23	190	1200	34		
			2300		23	
			1290		42	
			1850		77	44
9	23	176	3300	41		41
10	23	160	850	61		
			1320		33	
			1550		55	
			1150		45	48.5
11	28	160	3650		18	18
12	29	165	940	29		29
13	25	155	2650	33		33
14	29	175	2050	50		
			1500		33	41.5
15	28	165	—	37		37
16	29	180	1200		28	
			—		38	33
17	32	160	1030		24	24
18	27	160	930		25	25
19	27	180	1920		44	44
20	26	155	1580		18	
			1600		19	18.5
Average				40.5	35.1	37.8

TABLE 2. PITUITARY AND PARASELLAR TUMORS

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*
Age	21	24	41	48	41	53	60
Weight, lb.	195	195	170	148	200	245	162
Height, in.	70¾	70	63	71½	71	73	71
Clinical features	Headache most of life. Blurring vision in left eye 1 yr. Retrobulbar neuritis. Cerebral type obesity	Headache 10 yr. Convulsions for 3 mo. Girdle obesity. Pathologic diagsis of oligodendroglioma of third ventricle. Pituitary showed relative decrease in acidophilic cells.	Diabetes requiring 70 U insulin daily. Partial optic atrophy. Right homonymous hemianopsia. Adrenals, thyroid and pancreas normal on pathologic study. Right petrous ridge meningioma with parasellar extension.	Headache, blurring vision and left hemiparesis. Arrested Marie-Strumpel arthritis. Pathologic diagnosis (postmortem) chromophobe adenoma of pituitary.	Mild, slowly progressing acromegaly for 10 or more yr. Headaches for 3 yr. No changes in visual fields or ocular fundi. Had roentgen therapy to pituitary and surgical removal of adenoma of pituitary 3 yr. ago.	Pain behind left eye for 2 yr. Intermittent cerebrospinal fluid rhinorrhea 4 yr. Blurring vision 2 yr. Right temporal hemianopsia found. No signs of acromegaly. Obesity is familial (The assay result of 59 was after roentgen therapy to pituitary with symptomatic improvement.)	Dull frontal headache 2 mo. Visual fields contracted. Pallor of left temporal optic disc.
Secondary sex characteristics	Scant beard and body hair. Fine skin	Scant beard and body hair of female distribution. Mild gynecomastia.	Normal	Scant beard and body hair of female distribution. Lack of libido and potency for 10 yr.	Normal	Normal	Normal
Genitalia	Subnormal size, partial impotence.	Penis subnormal in size. Testes small (1½×1 cm.) Prostate ½ normal size. Not impotent. Aspermia.	Normal at physical and postmortem examination	Normal	Normal genitalia, partial impotence 3 yr., worse after operation.	Normal except complete sexual impotence for 3 yr.	Normal except relative impotence.
Roentgenogram	A 5 cm. calcified tumor arising above and to left of sella, destroying sella and eroding into sphenoid sinus.	Erosion of sella, some calcification in suprasellar region.	Sella enlarged	Erosion of sella and convolutional atrophy due to increased intracranial pressure.	Sella turcica large with depressed floor. Acromegalic skull.	Sella turcica large with depressed floor. Posterior clinoids eroded.	Destruction of posterior clinoids. Depressed floor of sella turcica.
Glucose tolerance	Normal	Normal	—	Increased tolerance	Normal	Normal	Normal
Basal metabolic rate	−17%	−18%	−7%	−22% −13%	−7%	−9%	−8% −9%
Friedman test	Negative	1. Negative 2 Positive	—	Negative	Negative	Negative	—
Androgen assay, I.U.	18	Normal by method of chloroform extraction[1]	15	22 33	33	30 59	6 9

[1] McCullagh, E. P., D. R. McCullagh and N. F. Hicken: *Endocrinology* 17: 49. 1933.

(*d*), Fröhlich's syndrome, 6 cases; (*e*), diabetes insipidus, 1 case; (*f*), pluriglandular disease, 2 cases.

Tumors of the pituitary and parasellar regions. One chromophobe adenomata of the pituitary, 3 pituitary tumors of undetermined type, and 3 parasellar tumors are presented together (table 2) because of the positive evidence of a tumor. In *case* 1 and 3, there were evidences of mild prepuberal hypogonadism. The assay approached normal in *case* 1 and came within normal range in *case* 3 though the symptoms in each have been present for more than 10 years.

No reports have been found of assays made on urine of patients with parasellar tumors. Kenyon et al. (6) reported a case of pituitary chromophobe adenoma. In this case the urinary androgens were 4 I.U. per liter on an 11-liter collection. This value

TABLE 3. ACROMEGALY

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*
Age	20	30	42	47	29
Weight, lb.	200	210	193	210	161
Height, in.	75½	72¾	70	74	70½
Clinical features	Gigantism with hypogonadism	Acromegaly 5–6 yr. External strabismus. Optic atrophy. Homonymous hemianopsia.	Acromegaly 8–9 yr., still progressing. Large goiter.	Acromegaly 9 yr.	Early acromegaly. Bitemporal hemianopsia. Headache. 3rd nerve palsy—all relieved by x-ray.
Secondary sex development	Retarded mildly	Normal	Well developed	Normal	Normal
Genital development and function	Testes slightly below normal size.	Impotent. Left testes small. Atrophic prostate.	Normal. Impotent 2 yr. Potency normal since 1938.	Normal	Normal. Impotent 6 mo.
Blood pressure	136/86	108/70	190/110	118/72	120/80
Sella turcica	Normal	Enlarged	Enlarged	Enlarged	Enlarged
Glucose tolerance	—	Normal	Normal	Normal	Normal
Basal metabolic rate	−14%	−23%	+4 (2 tests 1934) +25 (10 tests 1938 and 1939)	−5%	−17% −3% −16%
Friedman test	Negative	2 negative	4 negative, 1 positive	Negative	2 positive, 8 negative
Androgen assay, I.U.	5.4	3.3	93.5 after roentgen treatment	36.8	High by method of chloroform extraction. Urinary androgens decreased after roentgen therapy.

was obtained by using the 2-hour hydrolysis, the equivalent for the 15-minute hydrolysis being 6.6 U per liter. Our patient excreted an average of 12 U per liter on 2 tests.

Acromegaly. Assays were made on the urine of 5 men with acromegaly, ranging in age from 20 to 47 years (table 3). Only one patient showed arterial hypertension. It is interesting to note that the measured amount of androgenic substance varies proportionately with the degree of clinical hypogonadism in these patients, but not with the degree of sexual impotence present. In the third patient impotence is associated with a high assay and in the fifth repeated assays made by the chloroform method showed a constant reduction in urinary androgens associated with a steady increase in sexual potence. Thus it appears in these cases that there are factors in acromegaly, other than those reflected in the urinary androgens, which are powerful agents in the control of sexual potence. It is interesting to note in a recent case of pituitary tumor proved by roentgen ray, that although urinary androgens were low, doses of testosterone propionate of 25 mg. 3 times weekly failed to relieve the impotence.

Cushing's syndrome. Two similar cases of Cushing's syndrome have been studied. It was observed that in 2 such similar cases, the first excreted negligible amounts of androgen while the second excreted quantities comparable to those in normal men.

Case 1. A man, aged 43, height 67 in., weight 241 lb., complained of nervousness, depression and poor concentration for 8 months. There was obesity of the trunk. The genitalia were normal. He was impotent and had no nocturnal emissions. The blood pressure was 195/110, the glucose tolerance showed a diabetic curve, blood cholesterol was 228 mg. per 100 cc., the B.M.R. ranged from +9 to −14%, the blood count was normal, and roentgenogram findings of the sella were normal. The diabetes subsided, blood pressure returned to normal and nervousness disappeared after roentgen therapy to the pituitary, and dietary

TABLE 4. FRÖHLICH'S SYNDROME IN ADULTS

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*
Age	19	19	24	24	47	31
Height, in.	66½	69	69	66	68	67½
Weight, lb.	170	188	241	217	220	184
Clinical features	Obesity, fine skin, subnormal psychosexual development.	Obesity, cerebral type, lack of energy, tapering fingers.	Girdle obesity, fine skin.	Girdle obesity.	Obesity, chiefly girdle, feminine behavior.	Girdle obesity fine skin.
Secondary sex development	High voice, scant body hair.	High voice, scant beard.	Beard scant, sexual function normal.	Scant beard and body hair.	Beard and body hair normal.	Normal voice, normal potency, scant body hair.
Genitalia	Penis subnormal size, bilateral cryptorchidism descent on antuitrin-S therapy before assay.	Subnormal size.	Subnormal size.	Normal.	Subnormal. Sperms 95% abnormal in morphology and non-motile.	Moderately subnormal size. Aspermia.
Sella turcica	Normal	—	—	Normal	—	—
Glucose tolerance test	—	Normal	Normal	—	—	—
Basal metabolic rate	−10% (6 tests)	−4% (2 tests)	−15% (2 tests)	−10% (2 tests)	−8% (2 tests)	−10%
Androgen assay, I.U.	15	0.1	11	34	8	42

management. All assays made prior to giving testosterone, were made by the method of chloroform extraction and showed very little or no comb growth. Assays carried out using benzol extraction showed 0.8, 1.6 and 0.0 I.U. of androgen, while the patient was receiving from 2.5 to 5 mg. of testosterone propionate 2 to 3 times a week. There was one positive and one negative Friedman test result.

Case 2. A man, aged 26, height 67 in., weight 142 lb., with history of onset of obesity 8 years ago, but who had lost weight recently, had had hypertension for 4 years and intermittent glycosuria for several years. He was known to have had an extensive and resistant fungus infection of the nails for 2 years. He complained of marked nervousness for several years. His face was round, fat and red, the trunk was obese with purple abdominal striae and the genitalia were small. The prostate was normal in size and hair growth was normal. Blood pressure was 170/100. The skeleton was markedly decalcified and one vertebra was collapsed. The glucose tolerance test showed a diabetic curve, the B.M.R. was −3%, roentgenogram showed that the sella was normal, serum calcium was 12.1 mg. per 100 cc. of blood and the serum phosphorus was 3.9 mg. per 100 cc. of blood. The Friedman test result was negative. The urinary androgen assay result was 39 I.U. Exploration of right adrenal showed a small adenoma which was not regarded as etiologic, and the removal of which was not followed by clinical improvement. After a course of roentgen therapy to the pitui-

tary he improved greatly, including the fungus infection of the nails, which was eradicated. At present his condition is almost normal.

Fröhlich's syndrome in the adult. This is a group of 6 cases of Fröhlich's syndrome in adult males having no proved lesion of the pituitary gland (table 4). It is seen that the average level of urinary androgens in these cases was distinctly below that of normal men for this age. *Case 6* showed the mildest degree of clinical hypogonadism and also the highest androgen assay.

Diabetes insipidus. One patient with diabetes insipidus has had assays. It was not possible for us to differentiate between a neoplastic and an inflammatory lesion.

Case 3. A man, aged 55, height 66 in., weight 152 lb., complained of frontal headache for 2 years with increasing frequency and severity, and polyuria for 4 months. Physical examination showed a normally developed man. There was a moderate hypertrophy of the prostate. The blood pressure was 158/98. The neurological examination gave normal findings. The B.M.R. was −7% and −12% on two tests. Eye examination and visual field examination on admission were normal. Findings of roentgenograms of the sella turcica and encephalograms were normal. He had some relief from the headaches and polyuria for about a month following spinal puncture and encephalogram. In 6 months he began to show some contraction of the visual fields and slight edema of the optic discs as well as a recurrence of the headaches and polyuria.

He was then given a course of roentgen ray therapy to the pituitary gland and all of his symptoms disappeared and have shown only a very mild recurrence after 8 months. The Friedman test result was negative before and after roentgen therapy. He had a partial sexual impotence prior to the treatment but sexual function subsequently returned to normal. The 24-hour urinary assay before roentgen therapy showed 15 I.U. of androgenic substance. This rose to 20 I.U. 1 month and to 29 I.U. 5 months after treatment.

Pluriglandular disease. The following 2 cases present evidence of pluriglandular involvement of unknown origin. It will be noted that the manifestations of endocrine disease are dissimilar in the 2 cases. An interesting feature is the normally developed penis and prostate in the first case, in whom there were no palpable testes and no demonstrable urinary androgens. It is not unlikely that the endocrine signs in this case are of central neurological origin. The sexual function and potency in either case is not known.

Case 4. A man, aged 21 years, height 69 in., weight 208 lb., had previously had a herniorrhaphy. Bilateral cryptorchidism existed. His behavior was awkward, dull and quiet, and there was obvious mental retardation. Hair of male distribution was excessive all over the body. The obesity was confined chiefly to the trunk. Testes were not palpable. The penis and prostate were of normal size. The blood pressure was 135/70. Findings of roentgenograms of head and sella turcica were normal. The glucose tolerance test showed a diabetic type of curve. The B.M.R. was −13%, plasma cholesterol 250 mg. per 100 cc. of blood. The Friedman test result was negative. Androgen assay result was 0.

Case 5. A man, aged 40, height 69 in., weight 401 lb. (his weight for the past 10 years), complained of headache and dizziness since head injury 9 months previously. His general development was not remarkable except the obesity which was uniform in type. The genitalia were normal in size. Blood pressure was 150/100. A moderate diabetes mellitus existed. The B.M.R. was −10%. The Friedman test result was negative. Androgen assay was 8 U.

Hypogonadism

Urinary androgen levels are presented in 27 cases of hypogonadism. All are well into adult age making these cases interesting for comparison with studies on normal men. On a basis of clinical evidence the cases have been classified under two main heads: first, 4 cases of prepuberal testis injury or atrophy and 12 cases of a functional type of eunuchoidism, which, on a basis of clinical study consisted of 8 cases of se-

vere degree and 4 cases of mild degree of eunuchoidism; secondly, 11 cases of hypogonadism occurring in adults, consisting of 5 castrates and 6 men in whom there was incomplete testicular damage or atrophy which had occurred after the individual had reached adult age.

In the 4 cases of prepuberal testes injury, (table 5) all showed distinct clinical evidence of eunuchoidism. The assays ranged from extreme low to normal.

The more severe cases of functional eunuchoidism (table 6), excreted from 0 to 23 I.U. daily, with an average of 3.6 or approximately one-tenth of the normal average. The 4 mild cases, excreted from 9 to 31 U with an average of 15 U.

Kenyon et al. (6) have reported 7 cases of eunuchoidism in which the urinary

TABLE 5. PREPUBERAL TESTES INJURY

	Case 1	*Case 2*	*Case 3*	*Case 4*
Age	25	21	37	18
Height, in.	66	68½	72½	70½
Span of arms, in.	82	83	76	73
Weight, lb.	107	134	160	136
Clinical features	Orchidopexy for bilateral cryptorchidism, age 15. Postop. testicular atrophy.	Testes injury at age 12.	Mumps orchitis at 13 yr. A herniorrhaphy at age 15 yr.; testes found atrophic and fibrotic.	Bilateral herniorraphy before puberty. Postoperative atrophy of testes.
Secondary sexual development	Eunuchoid proportions, puerile face, high voice, scant beard and body hair. Epiphyseal age 17 yr.	Eunuchoid proportions, puerile voice and facies, epiphyseal age normal.	Eunuchoid skeleton, puerile voice and facies, lack of secondary characteristics. Epiphyseal age 20 yr.	Eunuchoid body. High voice, no beard. Fine skin. Scant body, axillary and pubic hair. Epiphyseal age 13 yr.
Genital development and function	Penis aplastic, prostate minute, impotent, testes atrophic.	Bean-sized testes, undersized penis, small prostate, impotent.	Testes not palpable. Penis, scrotum and prostate very small.	Penis 6½ cm. long. Prostate rudimentary. Right testis pea-sized. Left testis not palpable. Underdeveloped scrotum. No erections or emissions. Semen exam. consists of 0.01 cc. of epithelial debris.
Roentgenogram of sella turcica	Normal	Normal	Normal	Not made.
Glucose tolerance	1. Lagging curve 2. Flat curve	—	Flat curve	Not made.
Basal metabolic rate	−7% to −14%	—	—	−13% −15%
Androgen assay, I.U.	2	16 14	33	18

androgen assays ranged from 7 to 33 I.U. per day by the 15-minute hydrolysis, with an average of about one-third of their normals.

Hypogonadism, castrates. McCullagh and Renshaw (7) found no comb growth-promoting material in chloroform extracts of the urine of 11 eunuchs. Kochakian (8), using a method of extracting concentrated urine with chloroform under reflux for 10 to 12 hours, found no comb growth-promoting substance in the urine of one castrate studied. Kenyon et al. (6) reported assays on 2 castrates. One, aged 21 years, completely castrated and suffering from schizophrenia, showed 1 I.U. per liter of urine on using the 2-hour acid hydrolysis. The other, age 56 years, was obese, effeminate, and showed a gynecomastia. The completeness of the castration was questionable. This patient excreted 3.5 U per liter of urine, the 2-hour hydrolysis method being used.

We present 5 cases (table 7), 4 of which were completely castrated and the fifth (*case 3*) gave a history that part of one testicle was left at operation. He is included in this group, however, because on examination no testicular tissue could be found

TABLE 6. EUNUCHOIDISM

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*
Age	27	29	30	31	33	38
Height, in.	68	71	68	67⅜	68	70
Span, in.	71⅜	71	75½	71½	67	76⅜
Weight, lb.	156	177	147	168	109	170
Clinical features	Eunuchoid figure. Appears to be about 17 yr. of age. Fine skin.	Complains of being nervous and fatigued. Obese with eunuchoid build. Fingers long and tapering.	Eunuchoid build.	Has grown 5¾ in. since age 21. Eunuchoid build.	Cryptorchid. Left orchidopexy at age 18 yr. Typical eunuchoid build. Appears to be 10 to 12 years younger than actual age.	Gray hair at 25 yr. of age.
Secondary sexual development	Very scant beard, axillary and pubic hair. High voice.	Almost no beard, axillary or pubic hair.	No beard, axillary or pubic hair. High voice.	High voice. No beard, axillary or pubic hair. Fine skin, lanugo type of body hair.	Fine pink skin, high voice. Scant beard, axillary and pubic hair.	Scant beard, axillary and pubic hair. High voice.
Genitalia	Penis 1 in. long. Scrotum undeveloped. No palpable testes. Prostate rudimentary.	Penis and 1 testicle about ½ normal size. Other testicle much smaller. Prostate very small. Occasional erection and nocturnal emission.	Penis and prostate infantile. Testes about the size of peas. Never any ejaculate.	Rudimentary penis and scrotum. Testes and prostate size of peas. Occasional erection.	Left testicle very small, right testicle not palpable. Scrotum underdeveloped. Penis 2¾ in. long. Prostate very small. Aspermia.	Scrotum not developed. Testes not palpable. Penis 2½ in. long. Prostate ½ normal size. No secretion or sperms. Some libido and potency after 25 years of age.
Roentgenogram	Sella turcica normal. Epiphyseal age 18 yr.	Sella turcica normal. Epiphyseal age 20 yr.	Slight enlargement of sella. Epiphyseal age 20 to 21 yr.	Sella shallow and small at 18 yr. Normal at age 31. Epiphyseal age 20 yr.	Epiphyseal age 17 yr.	—
Glucose tolerance	Normal	Normal	—	Increased tolerance	—	Normal
Basal metabolic rate	−7% to −24%	—	−21%	−18% to −27%	−14%	−19%
Friedman test	Negative	3 tests negative	Negative	Negative	—	3 tests negative
Androgen assay, I.U.	3 2 4	1 2	23 2 9	0 2 0 1 0 1	2	2 0

TABLE 6. EUNUCHOIDISM—*Continued*

	Case 7	*Case 8*	*Case 9*	*Case 10*	*Case 11*	*Case 12*
Age	60	66	20	20	20	43
Height, in.	67	66½	67	68	70½	68¾
Span, in.	72	63¼	—	—	72	—
Weight, lb.	150	147	128	135	131	193
Clinical features	Fine skin. Appears much younger than actual age.	Testes always small. Developed unequal gynecomastia last 4 years. Body not eunuchoid.	Grew 7 in. in height after 16 yr. of age. Fine skin. Masculine body contour.	Right testicular atrophy following mumps at 13 yr. of age. Recurrent tenderness in left testicle at 17 yr. of age. Cause undetermined.	Mild eunuchoid build. Complains of lack of energy.	Was considered a midget until 14 yr. of age. Normal masculine body build.
Secondary sexual development	No beard. Scant axillary and pubic hair. High voice.	Normal voice. Scant beard, axillary, body and pubic hair.	No beard. Very little axillary hair. Pubic hair about normal.	Scant beard. Pubic hair of feminine pattern. Normal masculine voice.	Scant beard, axillary and pubic hair.	Beard moderately scant. Skin, axillary and pubic hair normal.
Genitalia	Penis, testes and prostate much below normal size. Subnormal libido and potency.	Penis subnormal size. Testes size of peas. Prostate atrophic. Subnormal potericy.	Penis normal size. Testes subnormal size. Prostate near normal size.	Penis normal size. Right testicle small and atrophic. Left testicle and prostate subnormal size. Complete aspermia.	Normal size and development. Erections only occasional.	Normal size and development. Libido and potency always low. Impotent for past 3 years.
Roentgenogram	Sella subnormal size.	Sella normal.	Sella turcica normal. Epiphyseal age 16 yr.	—	Sella turcica normal	—
Glucose tolerance	—	Lagging curve	—	—	Increased tolerance	—
Basal metabolic rate	−9% to −16%	+1%	−9% to −20%	−5%	−8%	−18%
Friedman test	2 tests negative	—	Negative	2 tests negative	Negative	—
Androgen assay, I.U.	2 2 0	19	10	31 15	9	10

and he showed the body and emotional changes characteristic of castration.

Adult hypogonadism (testicular damage). The 6 other cases (table 8) represent various degrees of testicular damage or atrophy occurring in adults. The single assay results ranged from 3 to 19 U with an average for the group being 14.5 U which is less than one-half of the average for normal men. In such cases as are represented in this group with incomplete testicular damage, hypogonadism may be difficult to diagnose clinically since the symptoms are often not distinctive and the psychoneurotic elements confusing.

Addison's Disease

Several authors have studied urinary androgen excretion in cases of adrenogenital syndrome, and adrenal cortical benign and malignant tumors (6, 9, and our unpublished cases). By way of contrast, we felt it would be interesting to observe the urinary androgen excretion of cases of adrenal cortical insufficiency. Three cases of clinically proved Addison's disease are presented (table 9). All 3 patients showed normal genital and secondary sexual development. None complained of sexual impotence. There were no abnormal physical or laboratory findings other than those attributable to Addison's disease.

TABLE 7. MALE CASTRATES

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*
Age	44	52	52	64	63
Height, in.	63½	68	67	70	62
Weight, lbs.	150	143	145	188	142
Clinical features	Castration in 1928. Has had headaches, nervousness, depression, weakness, hot flashes, insomnia, loss of libido and potency since castration.	Castration 2½ yr. ago followed by hot flashes, nervousness, weakness and loss of libido and potency.	Left testicle removed in 1906. Part of right testicle removed in 1926, followed by weakness, nervousness, fatigue and impotence.	Castrated in 1934, followed by hot flashes, weakness, nervousness and loss of libido and potency. Prostatectomy.	Castrate for 7 yr. Normal sexual function prior to castration. Is emotionally unstable. No hot flashes. Complete loss of libido and potency.
Secondary sexual characteristics.	Fine skin, hair of female distribution on abdomen.	Normal	Fine skin. Pubic hair of feminine pattern. Scant beard and axillary hair.	Normal	Body almost hairless. Scant axillary and pubic hair of feminine distribution.
Genitalia	Complete castration. Penis normal. Prostate atrophic.	Complete castration. Penis normal. Prostate atrophic.	No palpable testes tissue. Penis normal. Prostate atrophic.	Complete castration. Prostate absent. (Previous prostatectomy.)	Complete castrate. Penis normal. Prostate atrophic.
Roentgenogram of sella turcica	Normal	Enlarged with elevated posterior clinoids.	—	Normal	Normal
Glucose tolerance	Normal	Lagging curve	Hypoglycemic curve.	Normal	Normal
Basal metabolic rate	−17% to −22%	−15%	−17%	0% to −12%	−23%
Friedman test	3 tests negative 1 test positive	Negative	Negative	4 tests negative 1 test positive	Negative
Androgen assay, I.U.	16 11	0	7	0	7 16 6 10 18

The sodium deprivation test as described by Power, Cutler and Wilder (10) showed a definitely excessive urinary excretion of sodium and chlorides in *case 1* and *2*. The test was omitted in *case 3* because of two previous crises. The first assay in *case 1* was made 3 days after beginning a low potassium diet, sodium chloride by mouth and 5 cc. of eschatin daily. The second assay was carried out on a specimen

TABLE 8. INCOMPLETE TESTICULAR DAMAGE OR ATROPHY IN ADULTS

	Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*
Age	41	41	46	50	52	62
Height, in.	66½	66	71½	70	71	62½
Weight, lb.	154	198	248	203	205	152
Clinical features	Bilateral mumps orchitis at age 16.	Complete impotence, atrophy of testes and gain of 20 lb. weight after bilateral herniorraphy 5 yr. ago.	Bilateral mumps orchitis. Obese. Decreasing energy and progressive impotence for 5 yr.	Mumps with orchitis at age 30. Obese.	History of mumps with orchitis.	Questionable history of delayed descent of testes in childhood. Married 17 yr. no children.
Secondary sexual characteristics	Normal	Normal	Normal	Normal	Normal	Normal
Genitalia	Penis, prostate and right testicle normal except chronic prostatitis. Left testis atrophic. Complete sexual impotence in past year.	Both testes atrophic. Prostate small. Penis normal. Small amount seminal fluid, aspermia. Impotent.	Penis and prostate normal. Atrophy of left testicle. Right testicle soft.	Left testicle small and atrophic. Right testicle normal. Penis and prostate normal.	Atrophy of right testicle, left testicle soft. Penis and prostate normal. Impotent.	Penis and prostate normal. Both testes subnormal size. Progressive impotence for 10 yr.
Basal metabolic rate	−8%	−18% −17%	—	−19%	+3%	−25%
Androgen assay, I.U.	17	19	19	3	13	16

TABLE 9. ADDISON'S DISEASE

	Case 1	*Case 2*	*Case 3*
Age	18	38	29
Weight, lb.	133	125	147
Average blood pressure	95/60	116/70	120/74
Chief symptoms and physical signs	Skin uniformly dark for past 6 yr. or longer. Pigmentation in oral mucosa. Moderate weakness. Loss of 12 lb. weight in last 6 mo.	Complained of fatigue, shortness of breath. Dull pain in lower abdomen most of time, nervous and weak. Oral mucosa pigmented. Skin normal.	Entered hopsital in severe crisis. History of nausea, weakness, hiccoughs; yellow skin and loss of 50 lb. Delirious. Skin and oral mucosa pigmented.
Duration of symptoms	Indefinite	1 yr. with little change.	1 yr.
Roentgenographic findings	Chest and adrenals normal.	Normal chest. Adrenals not visualized.	Calcified mediastinal gland and old fibrosis left chest. Calcification in both adrenals.
Sodium deprivation tests	Typical of Addison's disease.	Typical of Addison's disease.	Not made in view of crises.
Response to treatment	Great improvement in strength while on diet, added NaCl and eschatin. Blood pressure normal and marked gain in strength and in weight on desoxycorticosterone.	All symptoms definitely improved. Assay made after 3 mo. of treatment.	Nearly complete freedom of symptoms when assay was made after 3 mo. of of treatment.
Androgen assay, I.U.	10 45	20	30

collected 8 months after beginning treatment and at a time when the patient was on a low potassium diet and receiving 12 gm. of sodium chloride by mouth daily and 2 cc. of eschatin intramuscularly every second day. The assay in *case 2* was made 3 months after beginning treatment with a low potassium diet, added salt and daily injections of 4 to 5 cc. of eschatin or 5 mg. of desoxycorticosterone acetate. He was receiving the latter at the time of the collection of the urine for the assay. The specimen was collected from *case 3* after 3 months of treatment and at a time when the patient was receiving a regular daily dose of 4 cc. of eschatin.

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PERCUTANEOUS ADMINISTRATION OF TESTOSTERONE PROPIONATE FOR DYSMENORRHEA

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TESTOSTERONE PROPIONATE has been added to the armamentarium of the obstetrician and gynecologist, since this hormone has been found a valuable aid in the treatment of dysmenorrhea (1, 2) metro-menorrhagia (3, 4, 5), the menopausal patient (6, 7, 8), and the after-pains and painful engorgement of the puerperium (9). These papers deal only with parenteral administration of this hormone. The purpose of this report is to present our experiences with the percutaneous use of testosterone propionate in the treatment of dysmenorrhea with or without premenstrual tension (10, 11).

The therapeutic rationale for the use of testosterone propionate, in a disorder of the female may be briefly reviewed. All the sexual hormones possess bisexual properties, with wide individual variations (12). The female gonad, especially, possesses strong bisexual potentialities both on biological (13) and embryological (14) evidence. Moreover, normal human females may excrete as much androgenic substances in their urine as do normal males (15). Testosterone has been shown to be not only a powerful androgen, but also a very potent gynecogen,[1] particularly as regards its stimulative action on the female genital tract following castration atrophy (12). In short, testosterone may be as rightfully called a 'female' sex hormone as a 'male' sex hormone.

The gynecogenic activity of testosterone, however, is dual. On the one hand, it resembles estrogens in its ability to evoke premature opening of the vagina of the normal and castrate immature rats (16), to produce uterine growth in the rat (12) and cat (17), as well as to maintain the sexual skin (18) and stimulate the growth of the endometrium of ovariectomized monkeys (19). The concept that testosterone 'neutralizes' estrogens (20), therefore cannot be accepted as a mathematical plus-minus reaction. In general, testosterone and estrone exert a cooperative, synergistic effect upon the female genital tract (12). On the other hand, testosterone exhibits a progesterone-like action in its ability to elicit a progestational response in the endometrium of the rat (12), cat (17) and rabbit (21), as well as inhibit menstruation in the monkey (18) and man (4). In addition, testosterone can maintain pregnancy in rats castrated during pregnancy (22).

Like progesterone, testosterone will inhibit rhythmic intermittent contractions of the rabbit's uterus (23), as well as markedly decrease the tension at which air will persufflate the rabbit's tube (24). Motility of the human uterus and tube may also be diminished by testosterone (9, 24).

Most observers are agreed that the immediate cause of the pain in dysmenorrhea is associated with relatively excessive contractions of the uterus (25), producing a transient ischemia (26). Since testosterone will inhibit or markedly decrease intermittent myometrial contractions, its use in the treatment of dysmenorrhea becomes manifest. Clinical experience affords ample evidence of its effectiveness when administered parenterally (1).

[1] "Gynecogen," a collective term for all substances which are able to restore, to some extent, the female genital tract following castration atrophy, or stimulate its growth directly; or induce or maintain the secondary sex characteristics of the female.

Some of the sex hormones can be readily absorbed through the skin and mucous membranes. Percutaneously, estrogens have been demonstrated to be effective both in the laboratory animal (27), and in man (28). In a similar manner, androgens have been shown to exert systemic effects in the male both experimentally (27) and clinically (29). In the female, testosterone is also effectively absorbed, for we found that percutaneous administration of testosterone propionate would lead to the same precocious opening of the vagina of normal and castrate immature rats as obtained by subcutaneous injection.

About the same time these experiments were under way, one of our dysmenorrheic patients objected strenuously to the necessity for injections. Therefore, she was given the hormone dissolved in sesame oil and instructed to rub a little under each arm before retiring at night. The result proved to be so satisfactory that percutaneous administration has been prescribed for 9 additional patients.

PROCEDURES AND METHODS

Ten patients complaining of dysmenorrhea with or without premenstrual tension have been treated by the percutaneous administration of testosterone propionate in the last year and a half. At present the following indications for the use of this method have presented themselves: objection to the use of injections, young patients who were going to school and could not come regularly to the dispensary, those who had been treated previously by injection and who still had a few residual symptoms, and those who, having to leave town, could not (or would not) secure the injections. Each patient is strongly urged to receive parenteral therapy first, since the required therapeutic dosage by that route affords a guide to the amount necessary percutaneously. Moreover, we have found it to be a general rule that those who do not respond to parenteral therapy usually will not respond to cutaneous administration.

The dosage required by the percutaneous route is 3 to 6 times as great as that necessary by the subcutaneous route, depending mainly upon the vehicle. Sesame oil, in our experience, has proven more effective than any of the ointment bases tried. However, the ointment has a more or less greaseless base and so is preferred by patients.

The axillary region has been the one of choice on an empirical basis. As an alternative, the inner part of the thighs or the deltoid region has been utilized.

If gynecological examination reveals normal findings, the patient is told to start percutaneous administration 10 days to 2 weeks before the expected onset of the next period. However, if the uterus is found to be small or anatomically misplaced, or both, or if she is thirty years old or more, the patient is advised to start applications 4 or 5 days after the period is over and continue them for the remainder of the cycle. The total dosage per cycle has ranged from 20 to 90 mg. of testosterone propionate. It cannot be too strongly emphasized that each patient must be treated individually; therefore, no set dosage schedule can be outlined.

After 3 successive periods with little or no discomfort, therapy is discontinued for a month in order to note whether improvement is maintained. If not, therapy is repeated again.

There are several points to be noted in instructing the patient. She must be told not to use any of the so-called deodorants in the axillary region as these substances have been found to interfere very materially with effective absorption. Moreover, she is advised to shave off the hair in that area, since absorption is enhanced considerably by this procedure (30). It is suggested to the patient that she leave the tube or bottle containing the ointment or oil on her dressing table and use it when she retires for the night. Because of the therapy, the usual premenstrual molimina may very

frequently be absent. The patient, therefore, is advised to be prepared a few days before the expected onset of her period as she may be disagreeably embarrassed. The following case histories illustrate the method of approach.

Case 1. This case history provides some comparison of the intramuscular, subcutaneous, oral and percutaneous methods of administration. In addition, some study of the effect of concentration and vehicle on effective cutaneous absorption was attempted. The degree of dysmenorrhea was used as an end point.

A 23-year-old, white, divorced, professionally trained woman had suffered from severe dysmenorrhea ever since the menarche at the age of 13. Menses recurred at 28 to 32-day intervals and lasted 7 to 10 days, the flow being fairly profuse, with occasional clots, for the first 4 or 5 days. Her mother and one of two sisters also had dysmenorrhea. At the age of 11, an appendectomy had been performed. When she was 18 she married. At 20 she had a spontaneous abortion at 3 months of gestation.

Premenstrually, a severe dull headache would develop "all over my head" 14 to 15 days before the next period. It lasted 12 to 24 hours, being relieved with salicylates. About a week before the onset of flow, she noted a sharp increase in her appetite for all kinds of sweets and a craving for "lots of salt with my meals." However, on the day before the period began, anorexia and nausea appeared. Constipation, present for 2 days prior to onset, cleared up with the flow. Her face broke out into a mild acneiform eruption during the last week of cycle, clearing up spontaneously with the menses. Three days before the onset, her breasts swelled, became very tense and tender, while the nipples became extremely sensitive.

Approximately one week before the period began, there was a rather abrupt change in personality, From a quiet, retiring person, she became an extremely active, aggressive one. She was "always on the go," pursuing her duties with a zest that surprised her superiors. Her physical unrest was accompanied by a marked nervous irritability and emotional instability. Little things that she had not noticed a few days before, now tried her patience sorely. She rapidly lost her temper and cried easily if matters did not run smoothly enough for her. On the other hand, if all went well, she felt elated. However, the most outstanding symptom that appeared at this time was a remarkable increase in libido. Her desire for sexual intercourse, regardless of frequency of coitus, amounted to practically that of a nymphomaniac.

This manic activity would change the day before the flow started to a dull lethargic state, as though "I had burnt myself out." She now tired very easily, while her nervous irritability became almost unbearable, even to her closest friends.

An hour after the flow started severe pain appeared. This was described as a constant dull ache with occasional sharp shooting pains which radiated to the lower portion of the hypogastrium. The pain lasted 48 hours. She would have to go to bed with a hot water bottle and heavy sedation (codeine, or even morphine) to seek relief.

Physical examination and laboratory findings were essentially normal, except for a third degree retroversion of a normal sized uterus. Manual reposition failed. Surgical intervention was refused at this time.

In table 1, is outlined the effect of various methods of administration of testosterone propionates upon her symptoms. It is to be noted that although the premenstrual tension was completely relieved, her dysmenorrhea reappeared as soon as placebo therapy was substituted or treatment was discontinued. No effect was noted upon the premenstrual acne, or increase in appetite for sweets and salt. The premenstrual mastalgia was cured.

Three months ago, her uterus was suspended. Her last two periods have been painless. Her premenstrual tension is very slight.

Case 2. A 38-year-old nulliparous wife of a physician had suffered from severe dysmenorrhea since the menarche at the age of 12. Menses recurred at 28-day intervals and lasted 4 days, the flow being moderate the first 2 days and scanty the last 2 days.

Previous therapy had included pregnancy urine extracts (APL), several dilatations and curettages, estrogens, progesterone and irradiation of the pituitary. In addition, a third degree retroversion of a small uterus had been partially corrected with a pessary. These measures had given little or no relief.

Premenstrually, her breasts became full and tense 3 to 4 days before the onset. On the day before, she became moderately irritable. Her voice became husky. With the onset of flow, she experienced sharp intermittent lower abdominal pains which lasted 24 hours. She had to go to bed heavily sedated for relief. Nausea, anorexia, lassitude and marked fatigability were also present at this time.

Because of a small, anatomically misplaced uterus, and since she was over thirty, therapy was prescribed throughout the cycle. The first month she applied approximately 45 mg. of testosterone propionate dissolved in an ointment base containing 2 mg. of hormone per gm. of vehicle. There was definite decrease in pain, no sedation being required, although she did go to bed. The following month, the dosage was increased to 60 mg. of testosterone propionate. Pain was decidedly less, lasting 4 hours. Appetite was good and fatigue was slight. Flow was slightly increased in amount but not duration. The dosage remained the same for the next cycle. There was practically no pain although she went to bed out of force of habit. Mild anorexia was present. Therapy was discontinued to note any changes. The first day of the succeeding period found her out shopping. Since then (7 months), with no further therapy, she has had practically no pain with her periods. Slight premenstrual mastalgia, throatiness of the voice, and slight fatigability are the only residual symptoms.

Case 3. A young woman, aged 21, had suffered from very severe dysmenorrhea since the onset of menses at 13. Periods were regular, recurring at 30-day intervals and lasting 4 to 5 days. Pelvic findings were normal. She received 10 mg. subcutaneously in 2 divided doses during the week before her period and 5 mg. intramuscularly on the first day of the flow. The flow was painless. The following cycle she took 10 mg. of testosterone propionate, plus a bile salt tablet, orally, for the 10 days before her period. Again she had no pain. The next month she failed to report for treatment. This time she had pain. She was then placed on the ointment, applying 60 mg. of testosterone propionate during the 10 days before her period. She had mild achy feelings for the first few hours. Therapy was repeated the following month. This period was painless. Therapy was discontinued, and her pain returned again. Accordingly a pellet of crystalline testosterone propionate, weighing 25.6 mg., was placed under the skin of the thigh. (The results of the use of pellets will be reported in the near future.)

Case 4. A young woman 20 years old had mild dysmenorrhea and irregular periods until she was 17. At this time, an appendectomy was performed. Since then her periods have become regular but extremely painful, necessitating the loss of one or two days from work each month. Pelvic examination revealed a normal sized anteflexed uterus which was pulled over slightly to the right but was not tender to motion.

The first month she received 5 mg. of testosterone propionate subcutaneously 2 days before and 5 mg. intramuscularly on the day of onset of flow. There was some pain present on the first day, but with sedation, she was able to continue her work. The following month she took 10 mg. of testosterone propionate, plus a bile salt tablet, orally for 8 days before the onset of flow. There was some discomfort present but that was relieved with two additional 10 mg. tablets. The following month she applied the equivalent of 60 mg. of testosterone propionate in 10 days before the onset. There was no pain at all, except for mild pelvic discomfort. Therapy was repeated the following month. This time she had severe pain. Close questioning revealed that she had begun to use an axillary deoderant that month. This substance was discontinued. She used the ointment alone as before and had a practically painless period. The following month, 40 mg. of testosterone were applied with excellent results. Therapy was discontinued and she complained of moderately severe pelvic discomfort. At present 20 mg. of hormone in ointment form will relieve her whenever she has a return of pelvic discomfort.

Case 5. A girl 19 years old, had always had somewhat irregular periods, (26 to 40 days) accompanied by severe dysmenorrhea. She always received complete relief with 10 mg. of testosterone propionate given in 2 divided subcutaneous doses in the 7 to 10 days before her period. Whenever therapy was discontinued symptoms returned. She failed to receive relief with either the ointment or oil in doses up to 50 mg. of testosterone propionate.

This patient has been the only one, to date, who has failed to respond to percutaneous therapy after receiving relief by parenteral administration.

Case 6. A housewife, age 28, had always had severe dysmenorrhea. Menses were regular, lasting 6 to 7 days, and were moderately profuse. Pelvic examination revealed a slight retroversion of a normal-sized uterus. She had received 2 doses of 10 mg. of testosterone propionate subcutaneously during the week before her expected period, for 3 months. There was a complete absence of pain each time. She remained symptomless for several months, when a return of moderate pelvic discomfort at the time of menses recurred. She applied 20 mg. of testosterone propionate in sesame oil for 2 months, with complete relief each time. For the past 3 months, she has been symptomless.

Case 7. An unmarried teacher, 25 years old, had always suffered from severe dysmenorrhea, which often necessitated absence from school for a day. Menses were regular, normal in amount and occasionally contained clots. Gynecological findings were essentially normal. She had previously received relief with 15 mg. of testosterone propionate, given in divided subcutaneous doses in the 7 to 10 days before her expected period. With return of symptoms, she applied 40 mg. of testosterone propionate during the 10 days before her period, for two cycles, with complete success each time.

Case 8. A school girl aged 17, had had marked dysmenorrhea since she was 13. Her menses began at 12, recurred at 28 to 30-day intervals, and lasted 3 to 4 days. Severe cramp-like pains appeared about an hour before the flow began, and lasted 36 to 48 hours. As a result, she had to stay out of school one day a month. Gynecological findings were essentially normal. She had received complete relief for 4 months, with 2 subcutaneous doses of 10 mg. of testosterone propionate in the week before her expected period. Three months later, moderate pelvic discomfort reappeared. Accordingly, she applied the equivalent of 20 mg. of testosterone propionate for 2 successive periods. For the past 4 months, she has been free of pain.

Case 9. A married woman, never pregnant, aged 26, had always experienced very marked pre-menstrual irritability and emotional instability, since the onset of menses at 12. Her periods recurred at 30 to 32-day intervals and lasted 4 to 5 days. Starting 5 days before her period, her breasts became full, tense and achy. By the following day, she noted that she tired very easily and "was nervous and irritable." On the day before her period began, she became a "veritable shrew," according to her husband. Moderately severe dysmenorrhea was present for the first 36 hours of flow. Pelvic findings were essentially negative. Parenteral therapy (3×5 mg.) in the week before her period had given her complete relief for 3 months, both from her pre-menstrual tension and her dysmenorrhea. Pre-menstrual tension reappeared 2 months later, along with moderate pelvic discomfort at the time of flow. She applied 40 mg. of testosterone propionate for one month, 28 mg. the second month, and 20 mg. the third month, in ointment form, in the 10 to 5 days before her period, with complete absence of symptoms each time. For the past 2 months she has been asymptomatic.

Case 10. A young woman, aged 28, married, grav. I, para I, had always had moderately severe dysmenorrhea since the onset of menses at 11. There were no pre-menstrual symptoms, the pain appearing just before the onset of flow. Pelvic examination revealed a second degree retroversion of a normal-sized uterus. She received complete relief from symptoms with the application of 50 mg. of testosterone propionate in sesame oil in the week before her expected period, for 3 months. The fourth month she failed to menstruate. Friedman test 2 weeks later was positive. She recently was delivered of a normal, full-term baby boy.

DISCUSSION

Influence on absorption of the vehicle and concentration of hormone. Testosterone propionate was absorbed by the skin in clinically effective amounts. Critical study of the results obtained, especially in *case 1*, revealed that there are two cardinal principles to be considered in percutaneous administration of this hormone. These

TABLE 1. THE EFFECT OF VARIOUS METHODS OF ADMINISTRATION OF TESTOSTERONE PROPIONATE UPON THE PREMENSTRUAL TENSION AND DYSMENORRHOEA OF *case 1*.

Menstrual cycle	Treatment	Total dosage, mg.	Premenstrual breast tension	Emotional instability and nervous irritability	Nymphomaniac tendencies	Dysmenorrhea
1	None		Very painful for 3 days before onset	Quite marked; crying spells, temper tantrums, etc.	Very marked	Extremely severe for 48 hr. Required opiates, bed rest
2	*Intramuscular*, 5 mg. in 1 cc. of sesame oil 3 times during 8 days before onset of flow	15	Very mild	Mild, one crying spell	Moderately reduced	Very slight pelvic discomfort. No medication needed
3	*Subcutaneous*, 5 mg. in 1 cc. of sesame oil on 8th and 3rd days before menses	10	Absent	Extremely mild; slight irritability	Slight	No discomfort at all
4	*Percutaneous*, 0.5 cc. daily for 10 days before onset, 5 mg./cc. sesame oil	25	Absent	Still very mild	Much diminished, loss of libido	None
5	*Percutaneous*, sesame oil alone, 0.5 cc. daily for 10 days before onset	0	Very slight	Slight nervous irritability	Practically none	Very severe for 2 days. Nausea and vomiting. Required heavy sedation.
6	*Percutaneous*, 0.5 cc. daily for 10 days before onset 5 mg./cc. sesame oil	25	Absent	Very slight	Absent	None
7	*Percutaneous*, 2 gm. ointment daily for 10 days before onset 2 mg./gm. of ointment	40	Absent	Very slight; mild irritability	Practically none	Moderate discomfort, some nausea. Complete relief with 5 mg. intramuscularly
8	*Percutaneous*, 1 cc. daily for 10 days before onset 2.5 mg./cc. sesame oil	25	Absent	Absent	Absent	Slight pelvic distress, mild aching pain. No sedation needed
9	*Percutaneous*, 3 gm. of ointment daily for 10 days. 2 mg./gm. ointment	60	Absent	Absent	None	None
10	*Percutaneous*, 3 gm. of ointment daily for 10 days. 2 mg./gm. ointment	60	Absent	Absent	None	None
11	*Percutaneous*, cold cream alone, 3 gm. daily for 10 days before onset	0	Slight tingling at nipples	None	None	Very severe for 2 days. Required heavy sedation.
12	*Percutaneous*, 1.5 cc. daily for 10 days before onset. 2 mg./cc. sesame oil	30	None	None	None	Very mild discomfort. Relieved by salicylates
13	*Oral*, 10 mg. daily+bile salt tablet, for 7 days before onset	70	None	None	None	Very mild discomfort for few hours after onset
14	*Oral*, 10 mg. daily, plus bile salt tablet, for 5 days before onset	50	None	Very slight-felt "jumpy"	None	Moderately severe. Complete relief with 10 mg. intramuscularly 3 hr. after onset
15	None	0	None	Very mild irritability	None	Very severe for 2 days. Required much sedation
16	Retroversion corrected by surgical intervention	0	None	Within normal limits	None	None
17–18	None	0	None	Within normal limits	None	None

are the nature of the vehicle and the concentration of hormone per unit volume of vehicle. Sesame oil proved to be a more effective vehicle than the ointment base when the hormone was used in similar concentrations (compare menstrual cycles 8 and 12 with 7, 9 and 10, in table 1). Provided the total dosage remains the same, the greater

the concentration of hormone per unit volume of vehicle, up to a certain maximum, the greater will be its effective absorption (compare menstrual cycles 4 and 6 with 8 and 12 in table 1). In other words, 1 mg. of testosterone propionate dissolved in 0.1 cc. of vehicle will be much more effectively absorbed than 1 mg. dissolved in 1 cc. of the same vehicle (30). Other important points that should be mentioned are that absorption is more efficacious in a shaved area and that so-called axillary deoderants should not be used at the same time that percutaneous therapy is being employed.

The mechanism whereby the sexual hormones are absorbed by the skin still remains to be demonstrated. That the oily substances contained in the skin and secreted by the sebaceous glands play an active part seems obvious for it has been found that the best vehicles as regards the most effective absorption (in the rat) have been the common fat and sterol solvents—ether, alcohol and benzene (28, 30).

Percutaneous compared to parenteral and oral methods of administration. Comparison of the subcutaneous, intramuscular, oral and percutaneous routes reveals

TABLE 2. RELATIVE EFFECTIVENESS OF ADMINISTRATION OF A GIVEN DOSE OF TESTOSTERONE PROPIONATE BY THE INTRAMUSCULAR, PERCUTANEOUS AND ORAL ROUTES COMPARED TO THE SUBCUTANEOUS ROUTE. (Ratios based on clinical evidence from cases of dysmenorrhea, functional bleeding and menopause.)

Subcutaneous: intramuscular	1:1.5–2
Subcutaneous: percutaneous (sesame oil)	1:3
Subcutaneous: percutaneous (ointment)	1:6
Subcutaneous: oral (plus bile salts)	1:7+

that the subcutaneous route appears to be the most effective per unit dose (table 2). The relative values that are listed in this table must be regarded as only approximate since they are based on clinical evidence—an end-point difficult to assess with exactness. However, in menopausal patients, as well as in cases of functional bleeding, the intramuscular route has been found to be from one-half to two-thirds as effective per dose as the subcutaneous route (5), tending to confirm the observation noted in table 2.[2] That the cutaneous route is one-third to one-sixth as effective per dose as the subcutaneous one has also been reported for testosterone propionate in the male (29). The same ratio has been noted for estrogens in an ointment base (28).

When testosterone propionate is administered orally, the addition of bile salts considerably enhances its effective absorption. If bile salts are omitted, the clinical effects, in the dosage employed, are practically absent. This observation has been repeatedly confirmed in menopausal patients. In the latter, as well as in *case 1* (cycle 14), the oral route has been found to be approximately one-seventh as effective per dose as the subcutaneous one. Foss reports a ratio of 1 to 20 (29). However, he did not use bile salts.

Relief of dysmenorrhea. The manner in which the dysmenorrhea is relieved will not be fully clear until it is definitely known what factors initiate the pain. The resultant alteration in the strength of the contractions probably plays an important if not an essential role, since most authorities agree that relatively excessive uterine

[2] Additional evidence is offered by the following case. A patient was found to be allergic to sesame oil only at the time of her menstrual period. If 1 cc. of sesame oil were given intramuscularly within 60 to 48 hours of the onset of menses, she developed marked urticaria and itching. If given more than 3 days before the onset, no symptoms developed. On the other hand, if sesame oil were given subcutaneously 5 to 7 days before the onset, urticaria and itching appeared. In other words, the duration of action of a subcutaneous dose was twice that of an intramuscular one.

contractions are the precipitating factor. The immediate cause of the pain is believed to result from transient uterine ischemia produced by these contractions. That some women may suffer pain because of hypersensitivity to normal contractions cannot be denied (37). That testosterone propionate does relieve premenstrual tension is evident. Reasonably, then, the relief of the dysmenorrhea may be partly, or even entirely, caused by certain as yet unknown effects of testosterone propionate upon the nervous system. Why the relief is permanent in some people is still beyond explanation.

Excessive libido. An explanation for the nymphomaniac tendencies in *case 1* cannot be advanced nor the mechanism of its suppression be elucidated. A loss of libido was also noted in 3 cases of functional uterine bleeding treated with testosterone propionate (5). It is interesting to observe that the increased libido appeared in the luteal phase of the cycle. In guinea pigs, it has been noted that estrus is caused by the action of progesterone (31). Testosterone is capable of inhibiting estrus in guinea pigs (32) and rats (33). One must be very cautious, however, in applying animal data to such a complex factor as sexual drive in man. It is interesting to note that nymphomania, as part of the premenstrual tension syndrome, has been relieved with progesterone (11).

Premenstrual tension. The relief of the irritability and emotional instability must surely involve some action of testosterone propionate, either primary or secondary, upon the autonomic nervous system and the endocrines on one hand, and the psyche on the other. Just what the modus operandi may be, is still beyond the scope of our present understanding of these interrelationships.

Premenstrual mastalgia. The relief of the premenstrual breast tension cannot be explained on the basis of our present knowledge of the action of testosterone propionate upon the human breast (34). Clinically, this hormone has been found effective for the relief of this condition (1) as well as the painfully engorged breast of the puerperium (9).

Premenstrual craving for sweets and salt. The premenstrual craving for sweets has been explained on the basis of a premenstrual speeding up of carbohydrate metabolism (35). The desire for salt premenstrually may be associated with a premenstrual rise in estrogens and the presence of a corpus luteum. Estrone and progesterone may cause salt retention and lead to a positive water balance, thus explaining the premenstrual gain in weight of many individuals (36).

SUMMARY

Percutaneously, testosterone propionate has been shown to relieve dysmenorrhea and premenstrual tension. Percutaneous administration of this hormone involves at least two cardinal considerations. These are the nature of the vehicle and the concentration of hormone per unit volume. Sesame oil proved to be a more efficient vehicle than the ointment base used. Provided the total dosage remains the same, the greater the concentration of hormone per unit volume, up to a certain maximum, the greater the effective absorption.

The indications for percutaneous administration of testosterone propionate for dysmenorrhea with or without premenstrual tension are objection to parenteral therapy, maintenance therapy for residual symptoms after parenteral administration, and those to whom parenteral therapy is unavailable. Each patient should be strongly urged to receive injections first in order to determine response to testosterone propionate; the amount necessary for percutaneous administration may thus be gauged.

Dose for dose, the subcutaneous route is the most efficient method of administration. The intramuscular route is one-half to two-thirds as effective. Percutaneously,

testosterone propionate is one-third to one-sixth as effective as the subcutaneous route. Orally, combined with bile salts, it is about one-seventh as effective as when given subcutaneously. From a practical viewpoint, however, the percutaneous method is much more economical than parenteral therapy and so is preferable to the patient.

No signs of masculinization were ever noted. The only possible sign of defeminization was the loss of nymphomaniac tendencies in one patient.

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MAINTENANCE OF THE CORPUS LUTEUM FUNCTION IN WOMEN BY ESTROGENIC SUBSTANCES[1]

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A NUMBER OF experiments have been published in 1934 from this laboratory showing the relation between the function of the ovarian follicles and the development and endocrine activity of the corpus luteum (1). The investigations performed on rabbits led to the following main observations.

(*a*) If a mature follicle is allowed to remain after coitus while the surrounding ovarian cortex is destroyed by electro-coagulation, a normal corpus luteum does not develop from the follicle. There appears either a follicular or luteal cyst or a more or less atrophic corpus luteum.

(*b*) If the ovarian cortex around a mature corpus luteum is burned away in the same way, the growth and function of the corpus luteum is disturbed.

(*c*) If the experimental animal is treated with estrogen during these conditions, the development of the corpus luteum proceeds in a normal manner.

It was concluded from these experiments that the action of a certain amount of estrogen is necessary for the function of the corpus luteum. If the ovarian cortex which produces it is destroyed, luteinization is disturbed, but the injury can be repaired by administration of the follicle hormone.

Further investigations (2) into these questions showed that the administration of an estrogen prolongs the life and functional capacity of the corpus luteum in pseudopregnancy from the normal 14 to 18 days until at least 36 days. The uterine mucosa then varies in appearance. In some cases, the typical progesterone reaction disappears and the endometrium is proliferative. In other cases, the mucous membrane is more neutral, but occasionally the typical progestational proliferation remains. The reaction of the mucosa is largely dependent on the size of the estrogen dose given, but there are individual variations and, therefore, the type of the mucous membrane may differ widely in a series of animals, all of whom receive the same amount of estrogen.

These results agree in principle with the observations of Allen and Heckel (3) who also found that the life of the corpus luteum in pseudopregnant rabbits could be considerably lengthened by the giving of estrogen. There is one difference, however. Allen and Heckel seem to have always observed a progestational reaction in the endometrium, as long as they were able to preserve the corpora lutea by estrogen administration. This was not the case in our experiments, nor did Klein (4), who made similar experiments, always find progestational proliferation in the endometrium. The most striking change is the disappearance of the syncytium in the epithelium of the endometrium, and Klein raises the question whether the corpus luteum, despite its normal appearance, really functions. He admits the possibility, however, that estrogen, which is antagonistic to the corpus luteum hormone in

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some respects, is the direct cause of the disappearance of the specific progestational reaction in the mucous membrane.

In order to find out whether estrogen works directly on the corpus luteum or through the hypophysis, further investigations were carried out on animals hypophysectomized after coitus (5). Normally the corpus luteum of rabbits degenerates on the third to fifth day after hypophysectomy. In hypophysectomized animals treated with estrogen the corpora lutea remain for at least 14 days. The uterine mucosa then, as in the previous cases, presents a picture varying according to the strength of the estrogen dose and the individual case. In some instances the progesterone reaction persists, in others it is replaced by an estrous reaction, and in still others the membrane has a more neutral appearance.

Robson (6) later published identical results. There is thus no doubt that estrogen does not exert its action by way of the hypophysis, as Hidaka (7) maintained recently, but the follicle hormone acts directly on the luteal cells.

In a recent communication (8) Heckel and Allen showed that not only pseudopregnancy but also pregnancy can be prolonged in the rabbit by the administration of estrogen. When the delivery is delayed in this manner, the fetus dies probably as a direct consequence of the abnormal amount of estrogen introduced into the organism. In addition, it appears from Robson's investigations (9) that treatment with the follicle hormone may prevent the interruption of pregnancy which otherwise always follows upon hypophysectomy.

Certain other substances have been tested for their capacity to maintain the functional life of the corpus luteum without directly influencing the endometrium. A series of experiments on rabbits gave the following results.

The follicle hormone. Estrone, estradiol and estradiol benzoate have essentially the same effect. The life of the corpus luteum can be prolonged with all three. The appearance of the endometrium varies. After the same dose, progestational response is observed in some cases, but not in others.

Diethyl-stilbestrol. A series of experiments were made with the diethyl-stilbestrol described by Dodds and his coworkers. (Estilbin, from the Danish pharmaceutical firm A. B. Medicinalco, was used.) This preparation prolonged the life of the corpus luteum, both in pseudopregnancy and after hypophysectomy, in a manner analogous to that of the follicle hormone, as Robson also observed. The appearance of the mucous membrane varied in the same way as after the administration of the follicle hormone.

The corpus luteum hormone. It was of interest to learn whether the corpus luteum hormone itself was able to influence the luteal cells in the same way as the estrogenic substances, but such was not the case. The two preparations, lutex (Leo) and proluton (Schering), could not prevent the rapid degeneration of the corpus luteum after hypophysectomy, although 10 I.U. per day were used.

The male hormone. In consideration of the chemical relationship between the male and female sex hormones, tests were made with androsterone and testosterone. It was not possible to maintain the life of the corpus luteum with either of these substances.

The hormone from the suprarenal cortex. It was found that the cortical hormone, which is relatively closely related to the sex hormones chemically, did not exert any conserving influence on the corpora lutea.

Vitamin E. Judging from the investigations published, vitamin E seems to exert a direct or indirect action on the ovaries, and perhaps especially on the corpus luteum. For this reason an extensive series of experiments was carried out with relatively large doses of wheat germ oil, administered both by mouth and subcutaneously. No prolongation of the functional life of the corpus luteum in pseudo-pregnancy was observed to follow this treatment.

Vitamin C and vitamin D. These vitamins were likewise ineffectual in this respect.

Thus, of the substances yet tested, it is only the estrogenic which can maintain

the life of the corpus luteum. This is an unfavorable circumstance from the standpoint of therapy, because of the difficulty in knowing what influence they will have on the endometrium in a given case. A substance which stimulated the corpus luteum without disturbing the structure of the uterine mucosa would be a valuable asset. Until such a preparation is produced, it is best to limit therapy to the giving of estrogen in such small does that it does not affect the endometrium, in the hope that it will, nevertheless, influence the corpus luteum. Judging from the results previously published (10) on the treatment of habitual abortion, certain advantages are to be gained from this procedure, even if we still have no really objective means of judging its effect. Undoubtedly, it would be better if it were possible to increase the estrogen dose and at the same time administer the corpus luteum hormone so as to protect the endometrium. However, at present it is difficult to test this combination in a sufficiently great number of cases because of the financial burden involved in lengthy treatment with progesterone.

Heckel and Allen (8) stated that these observations have been made in rabbits only and that the observations cannot be applied directly to humans. No experiments have been reported which indicate that the administration of estrogen can prolong the functional life of the corpora lutea in human ovaries.

Zondek (11) stated that the menstrual cycle of women is definitely disturbed through the giving of large doses of the follicle hormone. Menstruation stops for a varying length of time because the development of the corpora lutea is inhibited and this in turn prevents the endometrium from going through the premenstrual stage, a fact which is easily determined by histologic analysis of the uterine mucosa. These conditions vary greatly from case to case, however, and the duration of the amenorrhea is not directly dependent upon the size of the hormone dose. Zondek considers that the inhibition is due to the estrogen causing a shifting of the proportion between the A and B hormone secreted by the hypophysis.

We have been able to verify Zondek's observation that estrogen treatment may lead to amenorrhea, in which the uterine mucosa exhibits no secretory phase. However, a case has been observed in which the mucous membrane was of the progravid or secretory type after estrogen administration, showing that the corpus luteum was stimulated by the follicle hormone to lengthened activity in a manner which agrees in principle with the observations made in animals. A second case has been observed in which an incomplete corpus luteum function was considerably improved by treatment with the follicle hormone. Since these two clinical cases appear of special interest, they will be further described.

CASE REPORTS

Case 1. The patient was a 37-year-old para III with regular menstruation. For a short time prior to entry she had suffered from mental depression and an itching eruption on the external genitals. On examination, the genital organs proved to be normal, except for signs of pruritus vulvae. The patient was treated with estradiol monobenzoate (ovex Leo). Ten thousand units were given intramuscularly twice a week for 10 weeks. During this period the menses ceased entirely and the pruritus disappeared. As soon as the treatment was discontinued, the normal menstrual cycle returned. A little over 6 months later, the pruritus recurred, and the patient was given another hormone treatment the same as before. Again the menses ceased, to return after the conclusion of the treatment. After about a year of freedom from symptoms, during which period the menses were regular, there was another recurrence of the pruritus and the same treatment was begun again. The menses stopped and 9 weeks after the last menstruation, when 18 injections had been given, a specimen of the endometrium was removed. It was found that the mucous membrane was in the secretory phase, showing tortuous glands, perhaps slightly wider than usual. Staining of the membrane

was positive for mucus. The stroma was loose, edematous in places, and decidual transformation was beginning. A few days after the specimen was taken, an apparently normal menstruation began.

Case 2. A 32-year-old nullipara complained of extremely profuse menstrual flow occurring at regular intervals for 4 years. The copious bleeding had caused her to become greatly anemic, despite treatment with iron, arsenic and liver preparations. Nor did curettage lead to any amelioration. The uterus was of normal size and the adnexa appeared normal. Treatment with estrogen was attempted. Six thousand I.U. were given daily per os during the second half of the intermenstruum. The menstruation then became fully normal and the anemia improved. The patient continued with the treatment for 6 months during which time the menstruation was of normal quantity. On her own initiative she then decreased the estrin dose to 3000 I.U. daily. The menstrual flow then became very abundant again, but returned to normal when the dose was raised to the amount originally prescribed. Further attempts to decrease the dose led to the same results. In order to find out more about the way in which the estrin worked, the patient was told to stop taking it for some time, and just before menstruation was expected, which turned out to be profuse, a specimen of the uterine mucosa was removed. It was found to contain wide glands and the cells showed only slight mucus production. The stroma was fibrous. The picture gave the impression of a slight tendency to hyperproliferation and only a slight secretion. The patient then resumed the estrin medication and before the next menstruation, which was normal, another specimen from the endometrium was taken. The endometrium then showed a typical progestational state with tortuous glands, lined with mucus-secreting cells and a loose stroma with beginning decidual transformation.

DISCUSSION

In the first patient the interval between the menstruations was lengthened by the relatively large doses of estrin. From the usual 4 weeks it was prolonged to about 10 weeks, or somewhat longer than the period of estrogen medication. Examination of the uterine mucosa shortly before a delayed menstruation showed the endometrium to have a typically premenstrual character and thus that the corpus luteum was functioning.

In the second patient, the estrogen had a favorable influence on the function of the corpus luteum, as far as can be judged. As the first specimen revealed, it was not functioning sufficiently to bring about a normal secretory phase. After the estrogen treatment, its function was intensified, with the result that the mucous membrane exhibited a typical secretory phase, and the patient's profuse menses became normal.

The amounts of hormone administered in the two cases were different. Relatively large doses were given in the first case and relatively small ones in the second. Nevertheless, the effect was much the same in both instances. This agrees with the results in the animal experiments. In some experiments distinct transformation was observed and in others it was not, although the estrogen doses were the same and the corpora lutea had a similar histological appearance.

The amenorrhea observed after treatment with the follicle hormone has been thought due to degeneration of the corpus luteum, but it is not improbable that it is sometimes due to a maintenance of the luteal function under the influence of the follicle hormone.

It is tempting to try to make use of the discovery of the follicle hormone's stimulating effect on the corpus luteum for therapeutic purposes in cases of menstrual disorders due to a deficient luteal function and also during the first months of pregnancy in cases of habitual abortion. However, the elaboration of such a therapy involves great difficulties at present, for the very reason that it is impossible to judge how the estrogen administered affects the uterine mucosa. If a premenstrual or de-

cidual reaction is prevented or disturbed by the administration of the follicle hormone, more harm than good is done, even if the corpus luteum is stimulated.

SUMMARY

A short review of previous investigations on the necessity of the follicle hormone for the length of life and function of the corpus luteum is given. Animal experiments have shown that the corpus luteum does not develop normally if the estrogen-producing follicular system in the ovary is destroyed. On the other hand, the period during which the corpus luteum functions in pseudopregnancy and after hypophysectomy is considerably prolonged by the administration of estrogen, as has also been pointed out by Allen and Heckel, Robson, Klein and others.

Different substances, including progesterone, androsterone, testosterone, the cortical hormone and vitamins C, D and E, were tested in animal experiments to see whether any of them had a stimulating influence on the corpus luteum without primarily affecting the endometrium. All these substances proved to be ineffectual.

Two cases are described which demonstrate that the follicle hormone has a stimulating influence on the human corpora lutea. In one of them the interval between the otherwise normal menstruations was considerably lengthened during estrogen treatment. A specimen of the endometrium taken shortly before a greatly delayed menstruation showed the mucosa to be in the secretory phase. The amenorrhea brought about by the administration of estrogen is thus not always, as previously assumed, caused by degeneration of the corpus luteum, but may also be due to a lengthening of its functional life.

In the second case the patient had profuse menorrhagia. The mucous membrane showed an incomplete secretory phase. After treatment with the follicle hormone, the menstruation became normal and the endometrium exhibited a typical secretory phase. The function of the corpus luteum was probably stimulated in this case as well.

It should be possible to use the follicle hormone for treatment in certain conditions due to faulty function of the corpus luteum. One disadvantage, however, is that the hormone is likely to cause disturbances in the endometrium.

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THE ASSESSMENT OF SKELETAL DEVELOPMENT OF THE HAND AND KNEE BETWEEN THE AGES OF 8 AND 14 YEARS[1]

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TODD'S ATLAS[2] of the development of the hand, and his description of the development of the knee as related to so-called bone age probably embodies the most minute and careful study of the subject that has been made. Within the age range where they are applicable, these hand and knee standards permit a more precise estimation of skeletal status than has heretofore been possible.

But on using Todd's data for assessment of bone age it seemed to us that some of the changes on which we were asked to make diagnoses were of very small degree indeed. It often was impossible to be sure within one or two steps (6 months' intervals). In order to discover the limiting critical sensitivity of the method we have studied 10 children over a period of 2 years in that age interval where diagnosis seems to us most difficult, namely ages 8 to 14. Left hand and left knee were taken on each of these children at 6-month intervals by as nearly standardized technic as could be conveniently managed.

The study started with one boy and one girl at ages 8, 9, 10, 11, and 12 and continued until each pair reached ages 10, 11, 12, 13, and 14 respectively.

Besides the x-ray exposures, one of us (L.M.B.) made anthropometric studies to assure herself that the children lay within the normal development expected for their age, and that they were of normal body type. For some of the children, accurate height and weight records extended back several years prior to this study.

Figures 1 and 2 show that all but one of the subjects continued throughout the period of observation well within the normal ratio of weight to height, the one exception being an overweight girl. As will be seen from the annotated ages at the end of their individual graphs, there was some variation from one patient to the other in regard to ratio of height to age. It is thought that these children were a fair sample of a normal, well-nourished population enjoying normal growth for their age.

The essential material at the end of the study consisted of 20 sets of 5 x-rays each: 10 sets of hands and 10 sets of knees; the elapsed time between successive x-rays in any set being very close to 6 months; the whole period involved being 2 years.

Labels were now covered up and each set of hands and each set of knees was shuffled within itself. Both authors then made independent efforts to arrange each set in chronological order, making use of every available skeletal evidence of growth. In some cases the appearance and development of a sesamoid, or the increasing size of an ossification center, carpal or patellar, or the ossification of the tibial tubercle

[1] Aided by the Rockefeller Fluid Research Fund.
[2] TODD, T. W.: Atlas of Skeletal Maturation, C. V. Mosby Co. St. Louis, 1937.

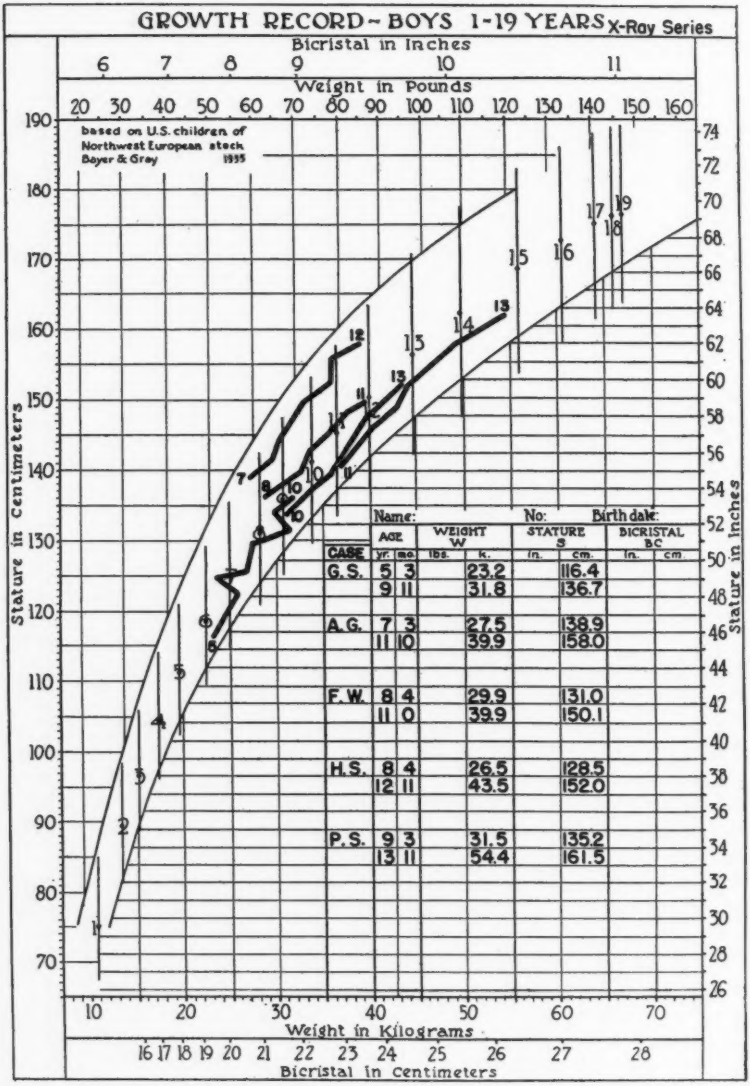

Fig. 1. Boys' growth graphs.

gave clues which were very much more usable for a single person than for general standardization.

Even so, when read by one of us, there was one instance of error in reading the hand and 5 errors in reading the knee. When read by the other, there were 3 errors in hand and 3 in knee.

Before evaluating the practical implications, two considerations are important.

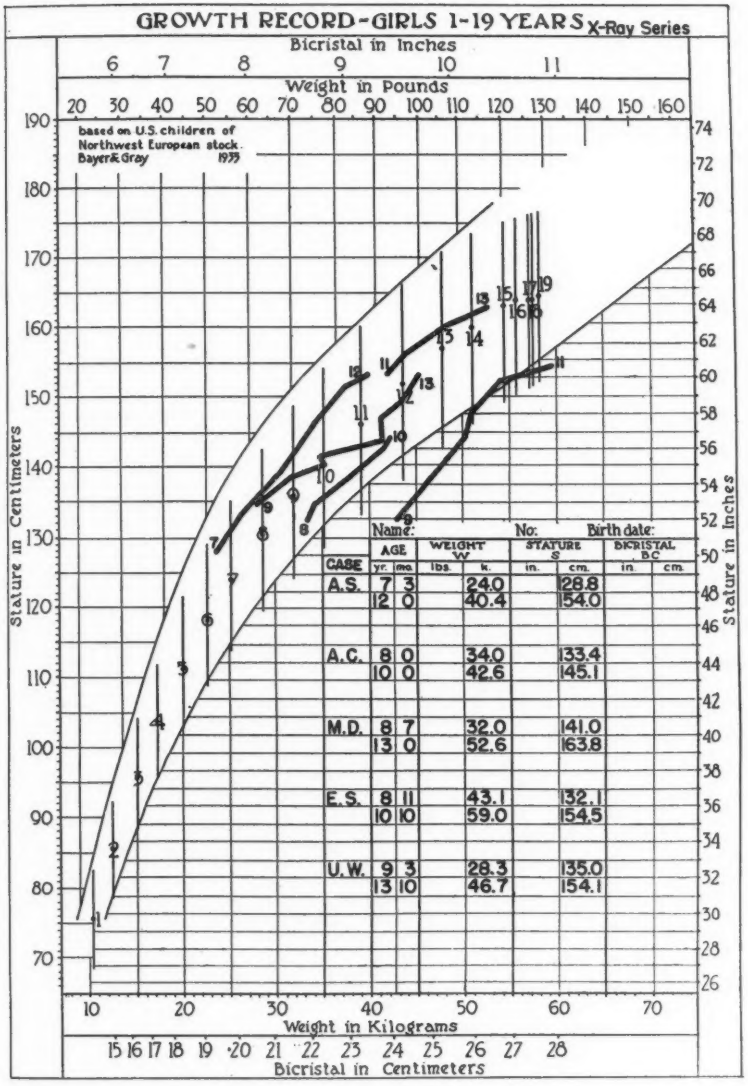

Fig. 2. GIRLS' GROWTH GRAPHS.

One is, that in matching x-rays of the same child against each other, greater precision of diagnosis can be expected than in the clinical laboratory, where the unknown x-ray is matched against a standard set. The other contrary point is, that in Todd's laboratory where the method was perfected, bone age is never assigned to a child by reading 1 or 2 joints, but by studying 4 or 5, the group of joints changing so as always to include the most rapidly developing joints for the age. However, for the age range 6

to 14, the wrist and knee are indicators as sensitive as any, and furthermore, there is not available to the general x-ray laboratory, a finely graduated scale for joints other than these.

SUMMARY

As a contribution to the problem of precision in bone age readings, a study was undertaken of the x-ray evidence of changes in hand and knee at 6-month intervals between the ages of 8 and 14 years. During this period, the diagnosis of bone age according to the methods outlined in Todd's atlas seems most difficult. Ten normally growing boys and girls were each observed by measurement and x-ray every half-year for 2 years, starting with a pair at ages 8, 9, 10, 11, and 12 respectively. The resulting 20 sets of hand and knee films could not, on their internal evidence, always be arranged in chronological order.

For this age range, therefore, we would argue that if developmental changes within the same knee and hand can sometimes not be detected after an interval of 6 months, then the assignment of bone age to a given joint under the usual conditions of a clinical laboratory can hardly hope to be dependable within limits narrower than a year.

THYROID GLAND DISTURBANCE AS A CAUSE OF UNEXPLAINED FEVER

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THERE ARE a certain group of patients who have a moderate rise in temperature (99–100°) and for which no explanation or cause can be found. These patients are usually suspected of having pulmonary tuberculosis, undulant fever, or a hidden focus of infection. The fever is often found during a routine employment or insurance examination. This group, as a rule, have no symptoms. Those who do have symptoms complain of fatigability, nervousness, irritability, insomnia, tenseness and a feeling of body tremor. To this may be added weight loss of moderate degree, such as 5 to 10 lb. Frequently these patients have had some infection preceding the onset of the fever. They are studied from all angles, including physical and laboratory examinations, without a definite diagnosis being established. In the physical examination there are three findings which were found to be common to all, namely, an enlarged thyroid gland, usually adenomatous in character; second, an increase in pulse rate, around 80 to 100 per minute; and third, a fine tremor of the extended fingers.

Of importance is the fact that the basal metabolism may be normal, a finding which is misleading. The practitioner, as a rule, usually dismisses from his mind the possibility of the thyroid as being a factor as the cause of fever.

That a toxic goiter may be present despite a normal or subnormal basal metabolic rate, at least as measured by our present day apparatus, has been reported (1–6).

Furthermore, the blood count, and in particular the leucocyte and differential count, is normal. The roentgen findings of the chest are normal. Other laboratory test results, such as those of cystoscopic examinations with normal roentgen findings of the genito-urinary system; rectal examination, blood Kahn test, blood sugar, nitrogen, urine and undulant fever tests are all normal.

The clue to these cases is the state of the thyroid gland. It is a well known fact that hypothyroid states, such as myxedema and cretinism, are accompanied by subnormal temperature. Hyperthyroid states, such as toxic adenoma and primary exophthalmic goiter, may have fever. Especially is this true of the thyroid storm in which hyperexia is present.

As previously stated, the fever frequently follows an infection, such as tonsillitis, influenza, or pyelitis, and the persistency of the fever for weeks and months later makes the physician quite certain that he is dealing with an infection which he is unable to locate. That the infection itself causes a mild activation of the pre-existing goiter is not seriously considered, especially in view of the normal B.M.R.

There is much evidence which shows that the thyroid gland, in its influence on the central nervous system, affects the temperature regulating mechanism. Ingram (7) has recently reviewed the experimental data pertaining to the hypothalamus and gives the experimental evidence which points to the hypothalamus and midbrain as the regulator of temperature.

If these areas are the central regulating mechanism for temperature, then the work of Schittenhelm and Eisler (8) is pertinent to the present discussion. These investigators made the important discovery that there is a local increase of thyroxin-

iodine in the interbrain by the experimental administration of the latter substance. They injected rabbits intravenously for 10 days, a daily dose of thyroxin equal to 1 mg. per kg. of body weight being given. The thyroxin had a selective action for the interbrain and the latter contained 1155γ of iodine. When 5 mg. per kg. of body weight was given orally in a single dose within 2 hours there was 1000γ of iodine in the interbrain, an increase above the normal content of over 100%.

Sturm and Schneeberg (9) found, after single intravenous injections of thyroid and iodine feeding of rabbits (1 mg. thyroxin per kg. of body weight), values in the tuber cinereum of 260 and 560γ of iodine, and in the pituitary between 700 and 2400γ of iodine.

The effect of the thyroid on the nervous system is, of course, well known and attention is called to the fact that the interbrain and the posterior lobe are composed of nervous tissue. It is also now well established that there are definite nerve connections between the hypothalamus and the posterior pituitary.

It is of interest that emotional upsets may cause a temporary rise in temperature ('psychogenic fever') and involvement of the hypothalamic temperature regulating mechanism may be inferred. Patients with toxic goiters are, of course, easily excited and frequently emotionally unstable. The goiter toxin affects the hypothalamic area in a deleterious way.

Perhaps the greatest and most frequent mistake made is to diagnose the condition as pulmonary tuberculosis. This is understandable because we have fatigability, excessive perspiration, rapid heart action, loss of weight and fever. Should the goiter, by chance, produce pressure on the trachea, and the symptoms of cough be added to the ones mentioned, then the diagnosis of pulmonary tuberculosis is advanced with more emphasis. Of course, sputum and roentgen examinations of the chest help to settle the diagnosis, but even in the face of negative findings the practitioner remains skeptical.

The proof that the thyroid gland is the cause of the fever is the fact that thyroidectomy in these patients results in the temperature becoming normal. This is usually accomplished within a week after thyroidectomy and the temperature remains normal thereafter.

Of course only patients who had symptoms such as previously mentioned were operated upon. The observations on these patients dates back over a period of 8 years and the results are gratifying.

SUMMARY

There are a group of patients who following an infection frequently have a low grade fever (99 to 100°). Some of these are asymptomatic, and some have in addition to the fever, fatigability, nervousness, irritability, insomnia, tenseness and a feeling of body tremor. The group herein referred to have an adenomatous goiter and its removal results in the temperature returning to normal.

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A CLINICAL CLASSIFICATION OF HYPOTHYROIDISM

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WITH PEOPLE IN GENERAL more interested in the endocrines, scant apology is needed for delving into the aspects of hypothyroidism, which have as yet been incompletely presented. It has been our experience that the frank states of cretinism and myxedema are presented by only a small proportion of patients suffering from thyroid deficiency. The clear-cut picture of cretinism and myxedema usually make their recognition simple. We are interested in the larger group that may go unrecognized, and in which permanent tissue changes often result. Although the cretin and the patient with myxedema are characterized by a depressed metabolism resulting in specific structural and functional changes, the cases we are presenting here show, in addition, a variable degree of reactivity of particular tissues. This multiform picture is the resultant of the physical status of the patient before this ailment and the changes due to the variable deprivation of the thyroid hormone.

In the past six years, there have been referred to the medical wards and the Endocrine Clinic of the 4th Division of Bellevue Hospital a large group of problem cases. From these we have chosen 60 individuals who, though complaining of widely diversified symptoms, have been definitely proven to be suffering from the lack of thyroid. A careful compilation of this data has made it possible to offer a classification which we feel is more comprehensive than any now existing, and which should aid in clarifying this important complex. All of our cases of hypothyroidism may be divided into four main groups.

Simple goiter. In this group should be classed patients having chronic thyroid enlargements with clinical or laboratory evidence of hypothyroidism. In addition to the increased demand for thyroxin during physiological cycles, there is the supply needed during exposure to cold, infectious disease and intoxications. It has been found that the thyroid may fail in adjusting itself to increased physiological demands. The resulting hypothyroid state through stimulation by a normal pituitary causes goiter. Morel remarked that goiter is one stage on the road to cretinism or myxedema. Goiter may be either sporadic or endemic. Numerous writers have traced the cause to lack of iodine in food or water. Alfalfa, cabbage and other substances have been found to be definitely goitrogenic foods. Continuous use of certain waters have resulted in goiter. Marine and Kimball (1) and others (2) have demonstrated the prophylactic and curative value of iodine.

CLINICAL CLASSIFICATION OF HYPOTHYROIDISM

I. Simple Goiter
   a) Sporadic
   b) Endemic
II. Primary
   a) Congenital
   b) Preadolescent
   c) Adult
III. Polyglandular
   a) Congenital
   b) Preadolescent
      1) Lorain-Levy Syndrome
      2) Fröhlich Syndrome
      3) Hypogonadism

III. Polyglandular (*Cont.*)
c) Adult
1) Exhausted Acromegaly
2) Simmonds's Disease

IV. Secondary
a) Operative or radiation
b) Exhaustive Graves Disease
c) Tuberculosis, lues, carcinoma and amyloid

Case 1. A.V., a girl, aged 13 years, came to our clinic because of swelling of the neck for which an operation had been advised. In her previous history it was noted that she had been a normal delivery. She had apparently developed normally physically and mentally until the age of 11 when started to menstruate. It was at this time that the enlargement in her neck was noted. Whereas she had always been plump and had been growing satisfactorily, with the onset of her menses there was no increased growth in height, with her increasing weight. Physical examination showed a somewhat undersized, overweight child. The face appeared puffy and firm. There was an acneiform eruption on her face and back. Hands were spade-like, fingers were small. The neck showed a diffusely enlarged soft thyroid with no bruit or thrill. Breasts were large. Otherwise the configuration was that of a well developed female. Laboratory findings were as follows: B.M.R. −25%; cholesterol, 220 mg.; E.C.G. showed low voltage; x-ray showed enlargement of the heart in all diameters; blood showed a moderate anemia; urine tests were negative; blood pressure 90/70. The patient was placed on Lugol solution, 5 minims t.i.d. In about 4 weeks she showed definite loss of weight and decrease in puffiness of face. Laboratory findings showed only slight change. She was then given thyroid, 1½ grains daily, and thereafter her B.M.R. rose to −3%, her cholesterol to 142 mg. and her E.C.G. and x-ray findings became normal. All hypothyroid stigmata disappeared.

Primary hypothyroidism. In this group are classed all cases in which structural and physiological changes can definitely be traced to a deficiency of thyroxin only. Clinical pictures in this group depend on the age of the patient when thyroid function first became inadequate. States of physical and mental retardation are dependent thereon. In the congenital group late walking, talking and teething are seen in conjunction with delayed physical development. However, the preadolescent group, in which the somatic element is not so deeply involved, tends to show more mental changes. In the adult forms there are gradations from clear cut myxedema down to those in which special tissue changes only predominate and the hypothyroid state is masked. Finally, whereas the congenital type usually shows marked growth impairment, there may be increased physical development due to compensatory hyperactivity of the anterior lobe of the pituitary. This experimental fact demonstrated by Rogowitsch (3) has been noted clinically (case to be reported) Niepce (4) also noticed enlargement of the pituitary in 5 cretins.

Case 2. *L.duP.*, a girl, aged 15 years, came to the clinic from the mental hygiene department. Because she had been crossed in some whim, she had slashed her mother with a knife. Previous history showed that she had been a normal delivery. There was no history of hereditary disease or endocrinopathy. She teethed very late, walked at the age of 4 and talked at the age of 5. She had never been accepted into the school system, even in the ungraded classes. At the age of 14 she started to menstruate. Physical examination showed a moderately overweight, undersized girl of 15 years who was completely mature as to her primary and secondary sex characteristics. Her hair was thick and her skin coarse and dry. There was definite evidence of striking mental impairment. Configuration was typical of a hypothyroid state. Laboratory examinations showed her B.M.R. to be −30%; cholesterol, 252; E.C.G., low voltage; x-ray of the chest showed enlargement of the heart. On thyroid therapy, 1½ grains daily for 6 months, her B.M.R. rose to −5%, her cholesterol to 150 mg. Her clinical picture also showed marked improvement. Whereas she had spent all day either eating or playing listlessly with a doll, she now would go out of doors alone and play with other children. She became self assertive and often disobedient. When thyroid medication was increased to 2 grains daily she became unmanageable, sneaked out of the house, took toys away from older children and when crossed in any way would inflict bodily harm

Fig. 1a, 1b. SIMPLE GOITER WITH HYPOTHYROIDISM. Note the facies and the thyroid enlargement. Fig. 1c, 1d. IMPROVEMENT OF THE FACIES AND RECESSION OF THE THYROID TUMOR. Fig. 2a, 2b. CONGENITAL PRIMARY HYPOTHYROIDISM. Fig. 2c, 2d. SOME IMPROVEMENT OF FACIES AND MENTAL STATE; slight if any other improvement in spite of continuous treatment. Fig. 3a. PREADOLESCENT PRIMARY HYPOTHYROIDISM. Fig. 3b. ONE YEAR LATER; WITH TREATMENT THERE IS CHANGE IN FACIES AND CONFIGURATION; BREASTS DEVELOPED, PUBIC AND AUXILIARY HAIR PRESENT. Fig. 4a. ADULT PRIMARY HYPOTHYROIDISM (myxedema). Fig. 4b. AFTER TREATMENT. Fig. 5a, 5b. CONGENITAL POLYGLANDULAR HYPOTHYROIDISM. Fig. 5c. TEN YEARS LATER. Some somatic and genital improvement. Slight if any mental advance in spite of continuous large doses of thyroid.

with whatever implement was handy. With medication dropped to a lower level she would revert to her previous amiable condition. After 7 years of continual thyroid treatment there has been no striking increase in growth or improvement in her mental capacity.

Case 3. J.P., a girl, aged 12 years, was sent to the mental hygiene department by the school authorities because she was considered subnormal. Her previous history showed that she had been a normal delivery. She had teethed, walked and talked at a normal age. She had been a plump, amiable, plastic child. Her brothers and sisters were normal. It was first

noticed when she entered school that she was unable to compete with her classmates. Her work continued poor and she did not play with other children. An observant teacher then suggested that she be sent to the mental hygiene department, which in turn sent her to the endocrine clinic. Physical examination showed a moderately overweight, undersized, child whose face and hands were puffy, skin dry, hair thick and coarse. She had never menstruated.

Fig. 6. PREADOLESCENT POLYGLANDULAR HYPOTHYROIDISM (Lorain-Levy syndrome). After 5 years of thyroid and hormone therapy there has been no somatic or sexual development, except the appearance of pubic hair. Fig. 7. PREADOLESCENT POLYGLANDULAR HYPOTHYROIDISM. (Fröhlich syndrome). Configuration unchanged with administration of thyroid. Fig. 8. PREADOLESCENT POLYGLANDULAR HYPOTHYROIDISM (Primary hypogonad). Symptomatic relief with thyroid and testosterone propionate. Fig. 9. ADULT POLYGLANDULAR HYPOTHYROIDISM (Simmonds's Disease). Secondary signs of hypothyroidism unchanged with therapy. Fig. 10a, 10b. ADULT POLYGLANDULAR HYPOTHYROIDISM (exhausted acromegaly). Fig. 10c. EIGHT MONTHS OF PITUITARY RADIATION RESULTED IN DISAPPEARANCE OF THYROID MASS AND THE DEVELOPMENT OF HYPOTHYROIDISM.

There were no signs of breast development, pubic or axillary hair. Laboratory data consisted of B.M.R., −25%; cholesterol, 245 mg.; E.C.G., low voltage; x-ray enlargement of the heart; moderate secondary anemia; urinalyses negative; blood pressure 85/70. Thyroid medication instituted consisted of 1½ grains daily. One year thereafter full development of her breasts, normal pubic and axillary hair, beginning menses, and regression of facies and configuration were noted. After 4 years of treatment there has been some increase in size, the patient has been menstruating normally, and her mental status is elevated to just subnormal.

This is another instance of a hypothyroid state passing unrecognized for many years. However, complete thyroid effect was impossible because of the long period of thyroid deficiency during her formative years.

Case 4. E.S., a woman, aged 32 years, entered the endocrine clinic with the complaint of coldness and sluggishness for a period of 5 years. She had been married for 8 years. There had been no pregnancies. Physical examination revealed the configuration typical of myxedema. Laboratory data showed: B.M.R., −35.5%; cholesterol, 260 mg.; E.C.G. low voltage; x-ray, marked enlargement of the heart in all diameters; normal urine; moderate anemia. Following thyroid treatment, 1½ grains daily, her B.M.R. changed to +7%, cholesterol to 165 mg.; E.C.G. and x-ray became normal. Menses became normal but sterility remained.

Polyglandular hypothyroidism. This group includes those patients in whom thyroid deficiency occurs with, or is secondary to, disturbed function of other glands. The age of inception of this insufficiency, superimposed on the endocrine configuration resulting from the primary gland affected, results in a complicated clinical picture. In Lorain-Levy infantalism the underlying cause is primarily hypopituitarism and secondarily the manifestations of thyroid insufficiency. As the hypothyroid state may intervene either early or late in this condition the resultant clinical picture can be variable. In hypothyroidism associated with Fröhlich's syndrome the picture may also become complicated. Thyroid medication in this type cannot alter the original Fröhlich configuration. This is equally true of the complicated primary hypogonad patient. In both of these types the duration and the state of pathological changes present before the thyroid involvement is a most important factor. In the adult it is only necessary to observe the picture of the exhausted acromegalic in whom a supervening hypothyroid condition complicates the tissue changes of the original condition. Pineles (5) reported 2 cases of acromegaly with myxedema of the skin, stupidity, and poor memory. There was distinct symptomatic relief with the administration of thyroid. Lastly we may place in this group cases of Simmonds's primary atrophy of the pituitary. All of these patients exhibit laboratory signs of marked hypothyroidism.

Case 5. J.S., a boy, 16 years old, was referred to the endocrine clinic from the orthopedic clinic. His previous history showed that he teethed, talked and walked very late. He had always been a plump, undersized and overweight child. He always had been amiable, plastic, but had never been admitted to school because of marked mental retardation. His brothers and sisters were normal. There was no history of hereditary disease or endocrinopathy. Physical examination revealed a boy of 16 with obesity, genital and somatic underdevelopment, with a cretinoid configuration. He had obviously had Perthes's disease of the hip. His skin was soft and hair soft and silky. Laboratory data showed: B.M.R. −40%; cholesterol, 220 mg.; E.C.G., lowered voltage; x-ray enlargement of the heart; slight secondary anemia. Urinalyses were negative and blood pressure was 95/70. Thyroid therapy with as high as 15 grains daily produced slight if any improvement. After 10 years of treatment there has been somatic improvement and genital development. The poor thyroid response postulates a polyglandular condition.

Case 6. M.D., a girl, 16 years old, was brought to the endocrine clinic because of a peculiar generalized skin eruption. Lesions had been of 1 year duration. Their size varied between that of a pin-head and ½ inch in diameter. Originally they were purplish and raised but gradually became flattened and pigmented brownish black. From her previous history we gathered that she had been a normal delivery. She had talked, teethed and walked normally. It had been noted that although her progress at school was perfect she manifested no interest towards her family or home. She was introspective and made no attempt to interest herself in normal contacts. There had been no history of physical or mental trauma. No history of relevant infectious disease was given. Physical examination revealed marked somatic and genital underdevelopment. Mental status was normal to slightly subnormal. Rectal examination showed a hypoplastic uterus. Laboratory examinations showed: B.M.R., −30%; cholesterol, 160 mg.; E.C.G., low voltage; x-ray showed enlarged heart, and delayed union of epiphyses; blood examination showed slight secondary anemia and increased sugar tolerance; urine tests results were negative. On thyroid medication there

was slight physical and mental improvement. However, with 5 years of treatment there has been no evidence of external sex signs except for pubic hair. Menses have not been established despite massive doses of estrogenic and gonadotropic hormones. At the age of 21 she still shows delayed union of the epiphyses, especially the radius. There has been no increase in skeletal growth.

Case 7. M.S., a boy, aged 14 years, entered the clinic complaining of overweight. Previous history showed normal childhood development. He had always been chubby. His mental status was average. He had had the usual childhood diseases. His family history was negative. Laboratory examination revealed his B.M.R. to be −24%; cholesterol, 198 mg.; x-ray, broadening of the transverse heart diameter; urinalysis results were negative and the blood picture normal. Thyroid medication, 4½ grains daily, caused some improvement in his mental alertness, a slight decrease in weight and a rise in his metabolism to −2%.

Case 8. F.F., a young man aged 26 years, entered the clinic complaining of mental sluggishness and poor memory. His previous medical and surgical history was negative. His family history revealed nothing abnormal in either his parents or siblings. He had always been tall and underweight. In the past few years he had noted a gradual loss of memory. He had had no libido but had practiced masturbation. Physical examination revealed a male with a long thin bodily configuration, arms and legs thin and long. There was no hair distribution on the chest; it was scant in the axillae and on the pubis. Genital examination showed long flaccid penis and small sized, almond shaped testicles. Rectal examination revealed a very small even prostate. Laboratory findings showed: B.M.R., −20%; cholesterol, 180 mg.; E.C.G., normal; urine and blood test results and x-ray findings were normal. Thyroid; 1½ grains daily, gave some symptomatic relief and caused the B.M.R. to change to −5%. Testosterone propionate caused an increase in the size of the testes and the appearance of libido.

Case 9. B.F., a woman, aged 38 years, came to the clinic complaining of weakness. Her previous history was of interest. She had started to menstruate at the age of 14 and had always been regular. She was a para IV, her last child having been born 5 years ago. Her symptoms date from this delivery. Since the birth of her last child her menses never returned. She noted a complete loss of libido, progressive loss of weight (50 lb. in 5 years), and marked weakness. With this she noted loss of axillary and pubic hair and a vitiligo, especially of the face. Physical examination showed evidence of marked weight loss and depigmentation of the skin. Mental status was definitely depressed. Laboratory findings showed a flat sugar tolerance curve; B.M.R., −45%; cholesterol, 214 mg.; urinalysis and blood test results negative. Thyroid therapy caused little if any improvement.

Case 10. E.H., a woman, aged 32 years, came to the clinic complaining of bitemporal headache for 2 years, loss of weight and amenorrhea. She had gradually noted an increase in the size of her hands, her facial contours and especially nose and lips. She had also noted complete loss of libido. The patient was a para IV with a normal obstetrical and gynecological history. Her previous medical and surgical history was negative. Physical examination showed a classical picture of acromegaly. X-ray showed a large sella with eroded floor and posterior clinoids. In addition, the patient had a large lobulated soft thyroid. She had soft moist skin, marked tachycardia and marked tremor of the hands. Blood pressure was 140/90. Vaginal examination findings were normal. There were no mulberry ovaries. Laboratory examinations showed: B.M.R., +32%; cholesterol, 120 mg.; perimeter fields showed bitemporal hemianopsia; sugar tolerance was decreased; E.C.G., normal; urine tests negative; blood picture and blood chemistry, normal. The patient received x-ray therapy to the pituitary for a period of 8 months following which a marked change in her previous contours was noted. With the disappearance of her soft tissue thickenings her face and hands appeared normal. With the return of the perimeter fields to normal her headaches disappeared. Menses and libido returned. The thyroid size decreased so that it was barely palpable. However as the signs and symptoms of hyperthyroidism gradually disappeared the B.M.R. dropped to −22%, her cholesterol rose to 214 mg., and a picture of adult myxedema appeared. Thus an overactive adenomatous pituitary resulted in a marked increase in size and activity of the thyroid. With exhaustion of the pituitary artificially induced by radiation there followed a depression of the thyroid with resultant myxedema.

Secondary. In this group are included that small number of cases due to known disease of the thyroid such as tuberculosis, lues, carcinoma and amyloid. These are few in number and importance. Often the diagnosis can only be made after operation or autopsy. All of these conditions have been known to result in a hypothyroid state. To these may be added that ever increasing group of hypothyroidism cases resulting either from radiation of hyperactive thyroids or their removal in intractable heart disease. The burnt out thyroid that receives no treatment may also be added to this group.

DISCUSSION

It has been demonstrated that the lack of iodine, either as a result of faulty diet or water, or due to increased physiological activity or pathological physiology may

TABLE 1. DISTRIBUTION AMONG REPORTED CASES

	Age	Number	Male	Female
Simple goiter				
	13 to 25	2	0	2
Primary				
Congenital	12 to 32	5	3	2
Preadolescent	11 to 25	4	2	2
Adult	32 to 65	19	6	13
Polyglandular				
Congenital	12 to 16	10	5	5
Preadolescent	11 to 30	11	6	5
Adult	45	2	1	1
Secondary	50 to 65	7	5	2
		60	28	32

result in hyperplasia and hypertrophy of the thyroid. This can only take place if the thyrotropic hormone of the anterior pituitary lobe is present. This anatomical enlargement is the physiological attempt to compensate for a deficiency of thyroxin, and is not necessarily a hyperfunctioning gland. Actually, it is often associated with marked thyroid insufficiency. It is in this group that iodine is of prophylactic and curative value. In *case 1* the disappearance of the thyroid mass and the changes in the facies after therapy have been noted.

In the primary group some difficulty in differentiating between the congenital and the preadolescent types may be encountered. Careful inquiry will always show that with the congenital type the individual talked, walked and teethed late, whereas with the preadolescent type the child was quiet, good natured and amiable, often plump and with long curly hair. Attention is first directed to this type at about the time of admission to school. Then they are found to be definitely below parmentally and their physical development is inferior to that of their companions. The sexual maturation of both types is rarely affected. External sexual development and menstruation usually occur at a normal age level. Somatic underdevelopment is most marked in cases in which the condition started early in life and remained untreated. Mental retardation is noted in all three types. Evidence of hyperfunction of the anterior pituitary resulting in somatic and genital hypertrophy, in an effort to compensate for hypofunction of the thyroid, was noted in one of our cases. Of great interest was the invariable disappearance of special tissue signs and symptoms with but small doses of thyroid. It was remarkable to note cure of deafness, solution of ichthyosis, improvement in anemia and relief of signs of cardiac failure when the hypothyroidism was treated. Where small doses of thyroid were not effective, the probability of hypothyroidism could be excluded.

The polyglandular division presents a more difficult problem. In the majority of the patients of this group gland inferiority may be accepted as being congenital, but variations resulting from the irregular appearance of signs and symptoms of their primary gland condition with superimposed thyroid inadequacy are numerous. In some all the glands may be involved. In others a typical configuration may only appear towards adolescence or even later. Discovery and treatment of the thyroid component is of paramount importance. It must be realized, however, that this treatment when complete will leave the patient with the typical configuration of his primary gland affliction. This will be noted in all the photographs of the cases in this group. Often in difficult cases, the laboratory data may be insufficient for diagnosis. In these cases the therapeutic test is final. Thyroid in small doses is effective in the primary type; in the polyglandular type larger doses are needed and the result obtained is dependent on the degree of thyroid co-involvement.

In the secondary group, the hypothyroid state resulting from ablation or radiation is most amenable to treatment. Some feel that in these cases the physician who makes judicious use of dried thyroid, is in a better position to act as metabolic regulator than a diseased gland.

Finally, we should like to record our impression of the effect of thyroid insufficiency on the human organism. During the formative period any degree of deficiency has a telling effect on both the physical and mental development of the individual. Once past this stage what does the lack of thyroid secretion do to the body? Does it cause, or is it conducive to, degenerative processes? Close observation of our cases during periods of increased, adequate dosage and complete stoppage of thyroid medication have led us to believe that as the thyroid supply dwindles the tissues go into a sort of hibernation or suspended animation. This is a somewhat reversible reaction. When thyroid is given the metabolism gradually picks up and goes at a faster tempo; when it is removed there is a gradual recession with a lowering of the tempo until it reaches the hibernation stage. If thyroid is withheld it will stay at this level until reawakened by further administration.

SUMMARY

The cases presented are representative samples of patients as they present themselves to the clinic. Through a study of the data we have been able to classify them into four main groups. We have noted that the diversity of the clinical pictures were dependent on four main factors: age of the patient when the hypothyroid state made its appearance; constitutional heredity; interrelation with other endocrine glands, especially the pituitary, and tissue sensitivity-skin, nervous system, subcutaneous tissue, cardiovascular tree and the erythron being the most sensitive to the lack of thyroxin. Finally we have noted that in the primary hypothyroid states small doses of desiccated thyroid were needed, whereas in the polyglandular types larger doses were necessary and the resultant clinical picture after complete thyroid effect is that of the patient before the intervention of the hypothyroid state.

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REPORT OF THREE CASES OF "CLINICAL" ADDISON'S DISEASE SURVIVING MORE THAN FIFTEEN YEARS[1]

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THE SURVIVAL TIME in Addison's disease apparently is showing a tendency to increase; at least more and more cases of prolonged duration are now being reported. Guttman (1), in a careful analysis of 566 cases collected from the literature prior to 1930, that is, before the modern methods of treatment were available, found that the average duration of life of patients with Addison's disease was 34 ± 4.4 months if simple atrophy of the suprarenal glands existed, and only 13.3 ± 2.5 months when the disease was tuberculous in origin. A case surviving 5 years a generation ago was unusual; one such case surviving 7 years while taking adrenal products was reported by Judson Daland in 1918, was quoted in Osler's text book and was referred to frequently in medical literature. A decade ago, at the time of publication of our book on Addison's disease, we had personally observed 6 cases surviving 5 years or more, including 2 cases, one of 7 years and the other of 12 years duration. In the last two decades a dozen or more cases surviving 10 years have been reported by various authors (Crowell, Scheult, Schlür, Withington, Kahn, Germaine, Phillips, Kiefer, Held, Conybeare and Millis, Wakefield and Smith, Scott, Medlar and Greene and Reuben Johnson). Phillip's case survived 13 years and Roloff's case 14 years. We wish here to report 3 cases from our own experience surviving 18, 17 and 15 years.

Two fundamental factors are concerned in the mortality rate, the degree of hormonal deficiency and the nature of the underlying pathology which in the past has been tuberculous in more than 80% of the cases. Increasing survival time in Addison's disease would seem to indicate a better insight into its nature and problems, as well as progress in the methods of management.

Progress in management has been made along several lines: (*a*) more effective substitution therapy with more potent preparations of cortical hormone and improved methods of administration; (*b*) recognition of the important rôle of sodium and potassium ions leading to the increased intake of the former and avoidance of the latter; (*c*) better care of tuberculosis in Addison's disease itself, to fewer complications and exacerbations; (*d*) improved management of syphilis and recognition in Addison's disease of the necessity of minimizing the specific treatment of syphilis, while emphasizing the specific management of hormonal deficiency; (*e*) improved general management in caring for patients with this serious affection.

However, the present 3 cases are presented not to emphasize the effects of therapy, but rather to indicate that certain cases may survive for a long period of years despite the absence of specific therapy.

It must be recognized that these patients may have suffered from a milder form

[1] Presented at the College of Physicians, Philadelphia, on October 23rd, 1939.

of Addison's disease than is generally encountered, for in some instances the disease is apparently less fulminating than in others. It must be remembered also that recovery has occurred at times (2). Our 3 cases have all probably been of the milder type with prolonged periods of remission. Nevertheless, they have each passed through crises which might easily have led to death had they not been wisely managed. The first case, due to tuberculosis, ran a relatively mild course and exhibited excessive pigmentation; the second, due possibly to lues or tuberculosis, or both, has received only a minimum amount of antiluetic treatment; while the third, though acute in onset and unmistakable in its clinical manifestations, cleared up in a relatively short time and so might represent an arrested if not a cured case of Addison's disease.

Naturally the diagnosis in two of these cases might be questioned. In *case 2* the diagnosis was established before the patient visited the Mayo Clinic and was concurred in by several physicians abroad whom the patient consulted. In *case 3* the clinical picture of Addison's disease was complete. In addition two members of the dermatological staff, after careful study, pronounced the pigmentation to be Addisonian in nature. If this case does not represent Addison's disease, at least it cannot be distinguished from it.

Case 1. A white male first visited the clinic in 1911, at the age of 38. This patient, who lived near Rochester, was under more or less continuous observation at the Mayo Clinic for the next 24 years, from 1911 to 1935. He survived Addison's disease for 18 years and was the subject of much clinical and therapeutic investigation. He eventually succumbed to generalized tuberculosis and to Addison's disease, in May, 1935, at the age of 62 years. At the age of 36 he had bilateral pulmonary tuberculosis with pleural effusion from which he made a satisfactory clinical recovery. Pigmentation was first noted in 1917 and this continued to increase throughout the next 5 years. Weakness and exhaustion first appeared in 1922, and initiated the active course of Addison's disease. An upper respiratory infection in January, 1923, was accompanied by severe and continuous pain in the loin and lumbar region.

On his visit to the Mayo Clinic in April, 1923, he was very weak and markedly pigmented (fig. 1), especially over the exposed areas of the body. He had not complained of nausea and vomiting and had lost only about 8 lb. in weight. The oral mucous membrane was unequivocally pigmented, and in the right posterior cervical region, a chain of enlarged lymph glands was palpated. A few crackling râles were heard at the apices of both lungs. The systolic pressure was 110 and the diastolic 80 mm. Hg. Roentgenological examination revealed definite evidence of pulmonary tuberculosis.

A diagnosis of Addison's disease with pulmonary and lymphatic tuberculosis was made and the Muirhead treatment[2] begun. Symptomatic improvement was noted at once and after about two weeks the patient insisted on returning to work on his farm. He did light work for about a year and then returned for treatment of the cervical adenitis. The lymph nodes were proved tuberculous by biopsy and were treated by excision and radium with complete satisfaction. With the aid of his son, the patient carried on with comfort all the work required on a farm of 80 acres. The blood pressure remained low, averaging 100/75 mm. Hg, the patient continued at all times deeply pigmented. He tired more easily than normal. This was his only complaint. He suffered from 4 attacks of upper respiratory infection, with exacerbation of the Addisonian manifestations, from which he recovered more slowly than normal. He continued for years to take epinephrin by rectum and the whole gland by mouth, though for 2 years (1930–32) he remained in good health without taking treatment of any kind. In 1932 he suffered a mild return of symptoms and developed a craving for salty food, especially salt herring.

In March, 1934, he began to fail in strength and gradually lost 20 lb. of weight. On May

[2] The Muirhead treatment consisted of hospitalizing the patient and administering epinephrin to the point of tolerance, by hypodermic injection, by mouth and by rectum 3 times a day, together with whole gland or dried cortex by mouth, 2 to 5 gr. 3 times daily.

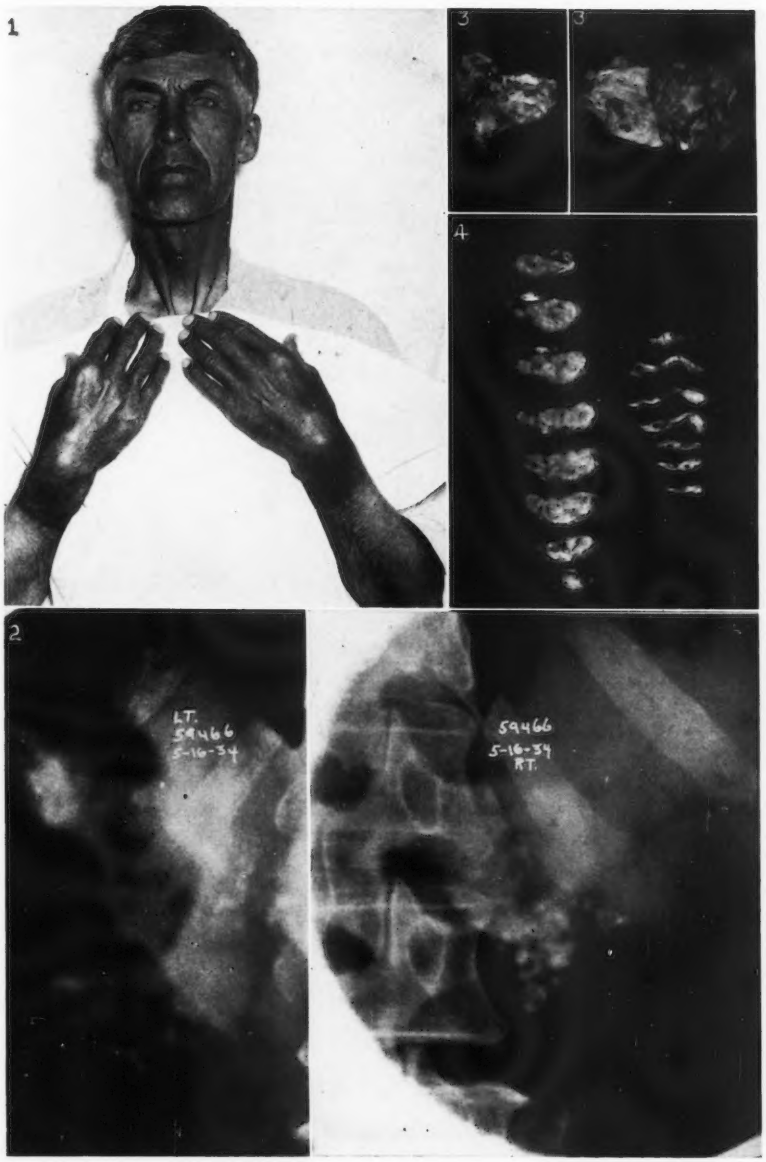

Fig. 1. *Case I.* Pigmentation in exposed areas in hands, face and neck. Fig. 2. Calcification of the adrenals as revealed by the x-ray. Fig. 3. Adrenal gland as removed at autopsy. Fig. 4 Cut surface of the adrenal glands as shown by repeated section.

Fig. 5, 6. Skin pigmentation of the neck and upper body, anterior and posterior view in *case 2;* an exacerbation of the pigmentation followed herpes zoster. Fig. 7, 8, 9. The intensity and distribution of pigmentation as seen in *case* 3 on admission to the Mayo Clinic, august 10, 1922. Fig. 10. Appearance of the patient in 1928 after the clearing up of any and all Addisonian manifestations.

11th he returned to the clinic complaining of marked weakness, mild confusion, loss of memory and severe constipation. Physical examination revealed marked pigmentation of the exposed surfaces of the body, the oral mucous membrane, pressure points and genitalia. The blood pressure was 100 systolic, 65 diastolic. The heart sounds were feeble but otherwise not remarkable. Evidence of old fibrous tuberculosis of the upper lobes of both lungs were noted. Roentgenological examination of the region of the suprarenal glands revealed extensive calcification of both sides. (fig. 2). At this time the blood urea nitrogen was 60 mg. per 100 cc. of whole blood, and plasma chlorides 544 gm. for 100 cc. The sodium was 310 mg. and potassium 19.3 mg. for 100 cc. of serum and the value of the total base was normal.

The patient was hospitalized at once and was given intramuscular injections of the suprarenal cortical hormone as prepared by Kendall. Sodium chloride, 10 gm. was given daily by mouth, supplemented by daily injections of 1 liter of physiological solution of sodium chloride. Within 24 hours the man felt much better and was up and about the ward. A prompt gain in weight was noted, due probably to retention of fluids, although his appetite and food intake had increased. Blood urea nitrogen fell to 24 mg. % and chlorides rose to 610 mg. % for plasma. There was no other change in the electrolytes of the blood. The man ate well, became much stronger and was promptly dismissed from the hospital and advised to take 10 gm. of sodium chloride daily but was not continued on cortical hormone. In the winter of the following year the pulmonary tuberculosis became active and the patient was sent to a sanitarium.

In May, 1935, the patient died and at the autopsy performed at the Mayo Clinic the following diagnoses were made.

(*a*), Chronic tuberculosis of adrenal glands with Addison's disease. 6.5 gm. (normal 15 gm.) Skin pigmentation III. (fig. 3, 4.)
(*b*), Chronic pulmonary tuberculosis upper lobe, obliterative pleuritis (left) and tuberculous bronchopneumonia.
(*c*), Emaciation III.
(*d*), Atrophy of pancreas (25 gm., normal 65 gm.)
(*e*), Chronic tuberculous pulmonary hilus and gastrohepatic nodes.
(*f*), Atrophy of pituitary (?).
(*g*), Arteriosclerosis I.

Comment. This may be regarded as one of the most interesting and remarkable cases of Addison's disease on record, not only because of its unusually long duration but because the patient has run the gauntlet of all our modern concepts of Addison's disease and has been subject to many studies and many forms of therapy. The patient was under continuous observation for a period of 25 years, being admitted to the clinic prior to the onset of Addison's disease and remaining under the care of the clinic until the end. He was first subjected to the Muirhead regimen, still later to the administration of large amounts of sodium, and finally to the cortical hormone prepared by Kendall. To each of these forms of therapy he responded satisfactorily. The case also indicates the important rôle which may be played by tuberculosis, which after a long period of latency became active and was largely responsible for his death.

This patient furnished an important clue as to the changes in Addison's disease in blood volume. Blood volume studies in 1923 revealed 83 cc. of blood and 51 cc. of plasma for each kg. of body weight. At this time he was extremely asthenic, his blood pressure very low and he was too weak to indulge in even mild degrees of activity. Yet there was no anemia at this time, according to the hemoglobin determination and the red cell count. Three months later with improvement in his physical condition the blood volume had increased over 1000 cc. and the plasma volume 700 cc. After a second interval of one year, blood volume had further increased 60 cc. The plasma volume remained about the same. Studies on this patient, among others,

led Rowntree and Brown in 1929 to write "It would seem from our studies that there is a relationship between the blood volume and the clinical status of the patient in Addison's disease. The patient in shock and collapse showed the lowest volume of blood according to body weight; improvement was followed by an increased blood and plasma volume. In other words, changes in the clinical status were associated with changes in the volume of blood plasma." The importance of these early findings has been markedly confirmed by all the recent studies dealing with the rôle of the suprarenal glands in maintaining hemeostasis, particularly in relation to plasma, electrolyte and water content of the blood and to capillary permeability.

Chemical studies of the blood were carried out, indicating the increased level of blood urea and the decreased level of chlorides in crises and correction by appropriate therapy. The electrolytes, sodium and potassium, however, were not changed materially, but as he represented an early case, there may be perhaps some doubts as to the accuracy of the methods.

Calcification of the adrenals was extreme, as indicated in the accompanying figure. The value of this roentgenological evidence was established during this patient's illness. In his case it finally determined the diagnosis, indicated the pathological basis as tuberculous in nature, and to a certain extent revealed the extensive nature of suprarenal destruction. Thus this patient seems to have played a definite part in the development of our knowledge of Addison's disease.

Case 2. A very tall thin man, aged 37 years, registered at the Mayo Clinic in June, 1926, complaining of progressive loss of weight and strength, increasing pigmentation of the skin and mild digestive disturbances for nearly 2 years. He had had syphilis, apparently with adequate treatment, in 1914. In 1921 the right middle figner had been amputated, ostensibly for tuberculosis. He had also had a painful abscess of the scrotum which had ruptured spontaneously. During the years 1924–25 he had lost nearly 60 lb., but had regained about 15 lb. during a period of improvement prior to registration. Physicians elsewhere who had seen him had considered the possibility of Addison's disease and had given him epinephrin with variable success. Iodine taken at one time gave pronounced temporary benefit.

On examination in June, 1926, the patient was deeply pigmented and asthenic. The blood pressures were 90 and 60. There was a nodule in the right epididymis with a small fistulous tract draining pus. The pupillary and deep tendon reflexes were normal. Findings of urinalysis, blood count and blood Wassermann reaction were negative. Roentgenograms of the thorax, kidneys, ureters and bladder revealed no abnormalities. A diagnosis of Addison's disease was made and the patient was sent to the hospital for treatment. He improved remarkably under the Muirhead treatment, and was dismissed with instructions to continue suprarenal cortex, 3 grains, 3 times a day, and epinephrin, 7 minims, 3 times a day, hypodermically.

In November, 1926 the weight had increased 16 lb., the blood pressures were 100 and 70, and the pigment had faded perceptibly. This decrease in pigmentation was confirmed by means of the tintometer. In November, 1927, the color was still lighter, but the blood pressure was unchanged. The patient was examined by a number of physicians, both in this country and in Europe, all of whom agreed that he presented the classical pigmentation of Addison's disease. In January, 1928, he was seen again; his weight and strength were still on the increase and he was able to do his usual work.

The patient visited the clinic again in 1935. He was in good general physical condition his weight was 170 lb. and his blood pressure 110 systolic and 70 diastolic. The pigmentation of his skin was unchanged and, in fact, there had been some marked additions to it following a recent attack of herpes. (fig. 5, 6). Nothing abnormal in the abdomen could be found. The heart and lungs were normal. Finding of urinalysis and blood counts were normal; the Kline and Kahn tests showed ± reaction. Roentgenograms of the chest showed an old healed primary complex in the upper left lung. Findings of x-rays of the adrenal glands were negative. Blood urea was 30, chlorides 594, sugar 70 mg. per 100 cc. and carbon dioxide combining power 52.2 vol. %.

A salt deprivation test was carried out over a period of 7 days without producing any significant signs of crisis and without any important changes in blood chemistry. During the course of the test the sodium fell slightly from 323.2 to 299.6 and the potassium from 18.7 to 16.3; total base from 152 to 143 mg. %.

He was dismissed with instructions to take 8 gm. of salt and 2 gm. of sodium carbonate daily. In response to my letter written in August, 1939, the following reply was received.

"Regarding my condition, on the whole I feel fairly well although weakness bothers me every now and then and although I do not lead a very active life, I still manage to get around fairly well.

"My pigmentation is almost normal except at the joints and belt line.

"My only medication consists of 2 sodium chloride tablets both morning and evening."

This patient is being placed on small protection doses of cortical hormone once weekly in the hope of preventing acute exacerbations or crises.

Case 3. A man, *J. L.*, aged 26 years, presented himself at the Mayo Clinic August 10, 1922, complaining chiefly of pigmented skin and weakness. He had had measles, mumps and whooping cough during childhood, pleurisy and empyema, with rib resection on the left side in 1900, pneumonia in 1906, and peptic ulcer in 1921. The onset of the ulcer had been sudden and associated with severe pain in the abdomen, followed by hematemesis and melena. He had been kept in bed on treatment for ulcer for 8 weeks. In February, 1922, he first noticed pigmentation; the face and neck gradually became darker, and now always appeared dirty. Later the genitals, anal regions, nipples and points of friction became diffusely pigmented and the skin peppered with small black spots. Illness had continued, with weakness and occasional spells of mild dizziness, particularly on straightening up suddenly. He said that the pigmentation had increased with exercise and had decreased following rest. He had had 5 intermittent attacks of pain in the left lumbar region. He weighed 129 lb. at the end of 9 months' illness, during which time he lost 39 lb.

At examination, the blood pressures were 94 and 60. The pigmentation was marked (fig. 7) and varied from brown on the arms to black on the genitals. There was a small area of pigmentation on the gum of the lower jaw (fig. 8). On the face and margins of the lips were small intensely pigmented areas (fig. 9). In the region of the ninth rib in the left axilla was a pigmented scar. Blood examination revealed the following: hemoglobin 80% (Dare), erythrocytes 4,700,000, leucocytes 4,300, lymphocytes 41, large mononuclear leucocytes 2, transitionals 5.5, neutrophils 48, eosinophils 3, and basophils 0.5. Gastric analysis showed a total acidity of 34, and free hydrochloric acid 18. The basal metabolic rate was −10. Findings of roentgenograms of the thorax and of the stomach were normal. A positive diagnosis of Addison's disease was made. The patient was seen in consultation by two dermatologists, Drs. Osborne and Goeckerman, who concurred in this diagnosis. The patient was sent to the hospital where he was given from 2 to 15 minims of solution of epinephrin 1:1,000 hypodermically, once or twice daily, 1 cc. in a solution of sodium chloride twice a day by rectum, and 5 grains of desiccated whole gland by mouth, 3 times a day. He occasionally suffered from nausea and vomiting. During the first few weeks the blood pressure ranged from 90 to 125 systolic and from 50 to 80 diastolic; on dismissal it was 130 and 80 respectively. The patient gained 20 lb., and improved steadily, and this was continued on his return home. Epinephrin was given hypodermically and by rectum to the point of tolerance.

A letter from the patient dated April 12, 1923 stated: "It seems to me that I am improving all the time and am much lighter in color than I have been for from two to three years. My wind is not what it should be, but I can see a great change for the better. I am taking the same amount of medicine prescribed by you last summer. I am quite active; sleep well eight to nine hours; I am still under the care of a physician." His physician reported at about the same time that the patient's weight was 145 lb., that his strength had not returned to normal entirely, but that his appetite was good and that he had indigestion occasionally. The blood pressure was 106/62; pigmentation had decreased but was still marked.

In September, 1926, the patient reported that he was in excellent condition. He was able to work all day in his greenhouse; his blood pressure was normal and his color good. He had a good appetite, and was able to sleep well. He was still taking epinephrin hypodermically

once a day. He had discontinued its use for about three weeks, but had worked too hard and become tired, so his physician had reinstituted treatment.

In October, 1928, the patient wrote that he was feeling well, and felt that he had improved continuously since his visit to the clinic 6 years before. His blood pressure had been normal during the last year, he had gained weight and was able to eat anything he chose. He reported that he was working long hours, but protecting himself as much as possible. A photograph (fig. 10) taken in 1929 shows the striking change in the patient's appearance, with complete disappearance of the pigmentation.

A letter to the patient in August, 1939, brought the following reply from his home physician, Dr. B. Coker, Durant, Oklahoma.

"Our mutual patient, *J. L.*, asked me to write you regarding his condition. I have been seeing him for the past twelve (12) years and except for occasional attacks of influenza he has enjoyed excellent health.

"The interesting points regarding his case are his weight remains around 165 pounds; his skin is fairly clear with very little pigmentation. His systolic blood pressure remains around 120, diastolic around 80.

"About four (4) years ago I found his urine contained sugar. I did a glucose tolerance test and found it to be in normal limits. I have had him on more or less restricted carbohydrates and occasionally he shows a one plus or two plus sugar in his urine. Yesterday he was in the office and the following findings were reported: Weight 162 pounds, skin clear, blood pressure 124/82, one plus sugar and one plus acetone in his urine. He states that he is feeling fine."

Under date of September 18th, 1939, the patient wrote as follows: "I have been feeling pretty good for a number of years: color good, weight about 170, appetite good and sleep well. So far as I can tell or remember I do not have any of the old symptoms that I had when you saw me in 1922 . . . I would like to have some of the pictures of knuckles and elbows that those artists made while I was at Mayo's. It would be interesting to compare them with the present coloring and see the contrast."

It would appear, therefore, that the Addison's disease in this patient has cleared up completely and that he represents an arrested, if not a cured case. It is possible that the injury to the suprarenal glands was functional rather than organic in nature. No evidence of tuberculosis was found at any time. Atrophy of the suprarenals seems excluded by reason of the complete restoration of function to normal.

SUMMARY

Three cases of Addison's disease surviving 15 years or more are presented. The first case, due to tuberculosis, succumbed after 18 years; the general tuberculosis playing a rôle in the fatal outcome, the local tuberculous lesions constituting the destructive factor in the suprarenal glands. The second case, due possibly to lues or tuberculosis, one or both, is still alive after having suffered for 15 years from a mild form of Addison's disease. The third case, idiopathic in origin and probably functional in character, represents an arrested case of Addison's disease which started some 17 years ago. These three cases represent the longest survival time in 116 cases discussed in 1931 in our book on Addison's disease. Dr. Snell informs me that still another patient of this group is alive and under observation at the Mayo Clinic, after 13 years.

These cases are presented not to emphasize the value of any of the old forms of treatment but rather to indicate that occasionally mild cases of Addison's disease can and do survive for very long periods of time, despite the lack of efficient and modern methods of management.

The author wishes to express his gratitude to the Mayo Clinic and especially to Dr. A. M. Snell for the privilege of recording the subsequent course of these cases, and to the American Philosophical Society for financial support from the Daland Fund.

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BILATERAL LOCALIZED LESIONS IN THE HYPOTHALAMUS WITH COMPLETE DESTRUCTION OF THE NEUROHYPOPHYSIS IN A PITUITARY DWARF WITH SEVERE PERMANENT DIABETES INSIPIDUS

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Occasionally there occur unexplained isolated lesions of the brain which, if investigated carefully from a clinical and pathological point of view, often aid materially in the understanding and the augmenting of the current knowledge concerning certain important functions of the human nervous system. Such discrete, well-placed lesions are often difficult or even impossible to reproduce experimentally because of their location and because of the associated tissue reactions which usually accompany surgical procedures. Investigation of such human cases often allows for a better understanding and interpretation of the results obtained from experimental studies.

CASE REPORT

C. L. (*H. N. 611653*) was an 18-year-old female. Her history indicated that her birth was attended with little difficulty. She had been healthy when born, began to walk at 10 months and talk at one year of age. She developed fairly normally, although it was noticed even in early childhood that the child was small in stature and had a moderate polyuria and polydypsia. During the first 11 years of her life her physical growth was very slow although intellectually she developed rapidly and had already completed her grade school studies. The polydypsia and polyuria increased so that at this time she was consuming over a gallon of liquid daily. At the age of 11 years, following a slight bruise to the head, the child began to complain of visual disturbance and was admitted to the hospital. Examination revealed a very small, chubby, well-proportioned and very intelligent individual who measured but 115 cm. in length and weighed 25.6 kg. She complained of a dimness of vision, especially in both lateral fields.

The general physical examination findings were negative. The only positive neurological findings were a bilateral optic atrophy, apparently of a primary type, and a bitemporal hemianopsia. The laboratory studies were normal. The findings pointed toward a lesion of the pituitary gland in the region of the optic chiasm. Since the patient had no objective complaints of headache, dizziness, or vomiting, it was deemed best to treat her conservatively rather than by surgery. She was, therefore, given a course of deep x-ray therapy over the pituitary region. Her vision, however, remained poor. She continued with her school work for another year, at which time she was forced to discontinue because of poor vision. The patient's findings remained unchanged until the age of 14 years when she began to have periodic spells of vomiting, sometimes projectile in nature and unassociated with any gastrointestinal pain or distress. At first these vomiting spells occurred about once a month and would persist both day and night for about a week. They increased in frequency during the next few years until they were occurring daily. Because of the increase in severity of the vomiting and the associated physical exhaustion, the patient was readmitted to the hospital at the age of 16 years. At this time she was somewhat dehydrated and mildly confused.

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Examination showed that her height had increased 4 in. during the past 4 years. She showed none of the changes of puberty; her menses had not begun. There was still the infantile distribution of pubic hair and her breasts were undeveloped. The neurologic findings were unchanged. The patient still had a bitemporal hemianopsia and a primary optic atrophy. There were no other neurological findings. X-ray findings of the skull were entirely negative. The spinal fluid was normal.

While in the hospital the vomiting spells gradually decreased in frequency and finally entirely disappeared. At this time the patient consumed as many as 4 gallons of water a day and had a most severe polyuria. She was again discharged and followed in the outpatient department. During the last 2 years of observation she continued to show occasional mild vomiting spells which cleared up spontaneously. At about this same time she also began to manifest some peculiar attacks. These would consist of periods of drowsiness during which she would sleep for days; when awake she was very easily fatigued, weak and tired. These spells of fatigue and drowsiness would continue for weeks and then clear up spontaneously. At times the patient would also have periods of elevated temperature, which were unmotivated and unassociated with any signs of infection. The hyperpyrexia would also clear up spontaneously.

While being observed in the outpatient department, her neurologic findings did not change. X-rays of the skull and sella showed no alterations. The laboratory findings were at all times normal. Her glucose tolerance was within normal limits. It was decided during this period to administer some of the pituitary extracts to this patient. Her length at this time was 121½ cm. from the head to the center of the external malleolus of the right foot. The patient was started with 1 cc. of a pituitary growth promoting hormone. This therapy was continued twice weekly for a period of 7 months, and the patient actually increased 2 cm. in length. The treatment was then discontinued for a short time and was not resumed because of illness of the patient.

The evening before her final hospital admission she suddenly developed severe pain in the abdomen and was admitted with a diagnosis of acute appendicitis. The appendix was removed and was found to be acutely inflamed. Following surgery the patient developed an extreme hyperpyrexia and died within 48 hours.

A complete postmortem examination was made. The body measured 130 cm. in length; the trunk measured 40 cm. from the suprasternal notch to the upper margin of the symphysis pubis; the lower limbs measured 72 cm. from the anterior superior spine to the base of the foot. There were but few significant gross somatic changes. The ovaries were about the size of those of a new-born child. The uterus and cervix measured 4 cm. in length. The uterus, itself, measured 2 cm. in length and appeared fibrotic. Microscopic studies of the ovaries revealed definite evidence of follicular activity with the formation of numerous Graafian follicles. Many of the follicles showed evidence of atresia. No corpus hemorrhagicum, luteum or albicans were present. Large areas of both ovaries were replaced by bands of connective tissue. The thyroid presented definite histological changes. Scattered amongst many large normal acini were others that were small, atrophic, and contained little or no colloid. In some areas the cells formed almost solid epithelial cords with no colloid, resembling the structure often seen in fetal adenomas.

The brain was carefully removed and injected prior to section. The vertex of the brain was normal in all respects. The posterior portion of the optic chiasm and the anteromedial segments of the optic tracts bilaterally seemed to be replaced by a cystic mass that projected downward from the anterior portion of the floor of the third ventricle. Sections removed from various areas of the cerebral and cerebellar hemispheres proved to be normal on histological study. The entire hypothalamic region was removed as one block and was cut and stained in serial sections with such stains as would facilitate the study of the individual cell groups, the myelin, the nerve fibers and the interstitial elements.

The tissue alterations within the hypothalamus were bilateral and were limited almost exclusively to those regions superior to and around the lateral aspect of the optic chiasm, extending laterally to involve also those areas adjacent to the medial portions of the optic tract. Although most of the tissue destruction was present in the lateral walls of the optic

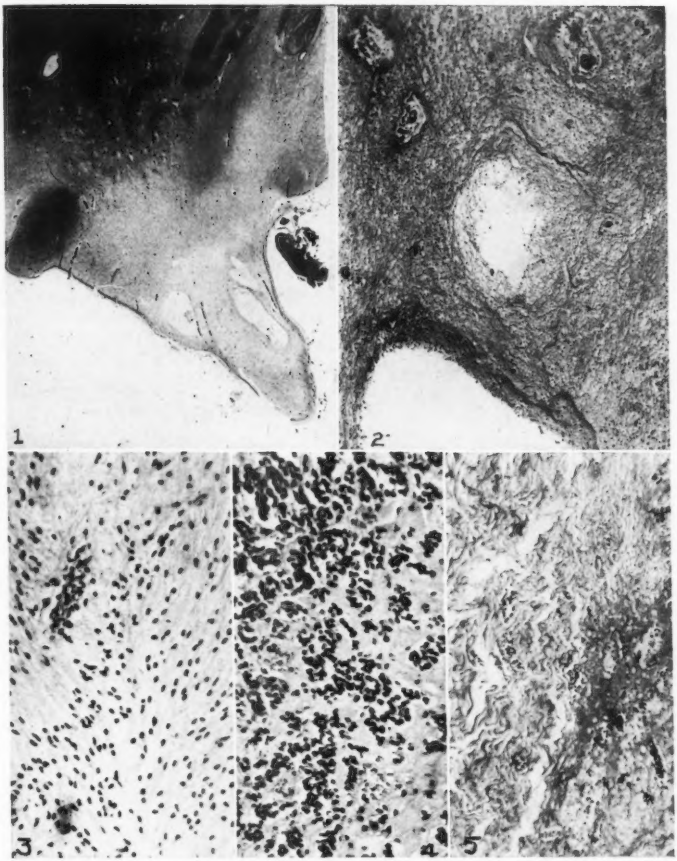

Fig. 1. SAGITTAL SECTION THROUGH THE HYPOTHALAMUS. The larger cystic cavities can be seen situated around the optic chiasm and extending a little posteriorly to involve the medial eminence of the tuber cinerum. Fig. 2. A HIGH MAGNIFICATION THROUGH ONE OF THE SMALLER CYSTS. Note the tissue fragmentation and vacuolization and the tendency for the cavities to be surrounded by dense acellular glial fibers. Fig. 3. AN AREA OF GLIAL PROLIFERATION WITHIN THE REGION OF THE INJURED SUPRAOPTIC NUCLEI. The macroglial proliferation is fairly extensive although the cell nuclei appear very uniform in both size and shape. Fig. 4. ANTERIOR LOBE OF THE PITUITARY GLAND SHOWING THE REPLACEMENT OF THE PARENCHYMA BY THE BANDS OF HOMOGENEOUS FIBROUS TISSUE. Fig. 5. A SECTION FROM THE NEUROHYPOPHYSIS. Note the complete replacement of all the normal elements by connective tissue. There are a few clusters of lymphocytes scattered amongst the wavy bands of fibrous tissue.

recess, there was some posterior extension of the pathological process into the antero-lateral walls of the infundibular recess involving the medial eminence of the tuber cinerium (fig. 1).

The most striking pathological changes consisted of extensive areas of demyelinization and tissue destruction which had resulted in the formation of many varying sized cavities (fig. 1). The larger cavitations were either partially or completely surrounded by dense acellular glial fibers, while the smaller lesions were usually less complete and presented only focal tissue fragmentation and vacuolization with little tendency toward secondary glial reaction (fig. 2). In some regions the tissue between and around these cavities showed an

almost complete diffuse demyelinization. This tissue appeared as a fine webby interlacing structure within which were scattered a few large nonproliferating astrocytes whose cell bodies could easily be made out due to the degeneration of the adjoining brain tissue. Active proliferation of the glial elements in this region was not apparent.

In many areas demyelinization around and between the large cysts was less complete and was accompanied by most extensive glial proliferation (fig. 3). This proliferation of the astrocytes ranged from a mild response to extensive reactions in which the cells were tremendously increased in number and varied greatly in size and shape. In many of these areas gliosis was prominent, often replacing much of the partially injured tissue. This macroglial reaction was also frequently nodular in type, producing well circumscribed groups of glial tissue. Fat granule cells or microglia were not observed in any of the sections. The blood vessels throughout and adjacent to the involved areas appeared intact. Some of the smaller vessels within the region of the injured tissue were surrounded by small mononuclear cells; many of these cells were oligodendroglia and microglia, but some definitely consisted of mesodermal elements and were no doubt small lymphocytes and plasma cells. No leukocytes were present in any of the sections.

The tissue destruction was almost strictly limited to the inferior portion of the pars supraoptica hypothalami in the region around the optic chiasm and optic tract. In none of the sections was any actual tissue destruction or glial reaction observed more superiorly in the region occupied by the paraventricular nucleus.

The various hypothalamic nuclei were studied in those serial sections stained with thionin. The supraoptic nucleus was completely destroyed bilaterally by the extensive demyelinization and gliosis. There were no cells in this region that could be identified as nerve cells. Even more striking was the almost complete absence of the cells of the paraventricular nucleus, even though no actual tissue injury had occurred in this region. Only a few of the typical large oval or rounded cells remained in the posterior portion of this nucleus. The rest of the hypothalamic nuclei seemed intact and in most cases could be identified.

The pituitary gland was removed and grossly appeared to be reduced in size. The entire gland, with the dura removed, weighed but 0.230 gm., which is about one-half the normal weight. Volumetric analysis by the paper-weight method from serial section showed: pars distalis (ant. lobe), 0.099 gm.; pars intermedia 0.002 gm.; reasonably normal appearing processes infundibuli 0.001 gm. and a densely fibrosed area (post. lobe), 0.128 gm. It appeared, therefore, that one-half of the hypophysis proper was composed of connective tissue. The anterior lobe was invaded and replaced by large bands of homogeneous fibrous tissue (fig. 4). In those regions where the anterior lobe cells were more or less intact, there was a reduction in the chromophilic cells. It was estimated that only about one-sixth of the normal number of anterior lobe cells remained. Even more striking were the changes in the posterior lobe of the hypophysis. Here there was a complete replacement by connective tissue of all the normal elements. Most of the connective tissue had undergone hyalinization, producing irregular wavy bands of tissue amongst which could be seen a few clusters of small lymphocytes (fig. 5). No normal posterior lobe could be identified. (We are indebted to Dr. A. T. Rasmussen for his most helpful advice and for the description of the changes in the pituitary.)

DISCUSSION

This case clearly helps substantiate and perhaps also augments some of the more recent work on the relationship between the hypothalamus and the neurohypophysis as well as the influence of these structures upon diabetes insipidus (1, 2, 3). Many investigators (4, 5, 6) have demonstrated a bundle of unmyelinated fibers which apparently takes origin in the supraoptic nucleus and passes to the neurohypophysis. The presence of such an intimate relationship between these two structures has been sufficiently verified experimentally by injury to the supraoptico-hypophyseal tract at various points between the supraoptic nuclei and the posterior lobe (2, 3, 7). In most cases there invariably occurred a marked atrophy of both the supraoptic nuclei and the posterior lobe. In very few cases, however, have the in-

vestigators succeeded in producing small discrete isolated lesions within the supraoptic nucleus alone, probably because of the mechanical difficulties involved. Broers (8) recorded one such lesion within a dog and Biggart (9) one in man. In our case, through some fortuitous occurrence, such bilateral isolated lesions completely destroyed the supraoptic nuclei. The effect upon the neurohypophysis was very striking, resulting in a complete destruction and fibrosis of this structure. These observations substantiate the experimental evidence already present, namely; that the cells of the supraoptic nucleus have definite neuronal connections to the pars nervosa of the hypophysis and that injury to one results in retrograde changes in the other.

Information concerning the possible relationship between the paraventricular and the supraoptic nucleus can also be deduced from our observations. Roussy and Mosinger (10), described fiber connections between these two nuclei and called these fibers the tractus intertangentioparaventricularis. These authors were uncertain as to whether all the fibers of the paraventricular nucleus ended in the supraoptic nucleus, or whether some passed directly into the stalk without stopping at the supraoptic nucleus. Experimentally, however, it has been fairly well established that very few fibers pass directly from the paraventricular nucleus to the neurohypophysis since injury to the latter or to the hypophyseal stalk, through which such a tract must pass, produced but little retrograde reduction in the number of cells within the paraventricular nucleus. Rasmussen (11) observed, in rats, a loss only in the cells of the ventrolateral or magnocellular portion of this nucleus, while Fisher, Ingram and Ranson (3) and Magoun and Ranson (2), observed a similar mild reduction in the paraventricular nucleus of cats and monkeys. In the latter animal it was estimated that about 20% of the cells of the paraventricular nucleus had disappeared. On the other hand, White and Heimbecker (12) produced hypothalamic lesions in dogs encroaching upon the tuber cinereum proper without actual involvement of the paraventricular nucleus and produced a marked loss of most of the cells of this nucleus. In our case, isolated lesions destroying the supraoptic nucleus, produced an almost complete disappearance of the paraventricular cells. The disappearance of these cells must be assumed to be due to some retrograde atrophy since there was a total absence of any other tissue changes in the region of this nucleus. These observations certainly lend support to the opinion that the paraventricular nucleus probably has most of its neuronal connections with the supraoptic nucleus and hence undergoes severe atrophy on injury to the latter.

The importance of the hypothalamico-hypophyseal tract to diabetes insipidus is also emphasized by the observations in the present case. The experimental and clinical literature on diabetes insipidus has been excellently reviewed and needs no further elaboration (3, 13, 14). Permanent diabetes insipidus has been produced in many animals by interruption of the hypothalamico-hypophyseal tract either at the supraoptic nucleus, in the pituitary stalk or in the posterior lobe itself. Fisher, Ingram and Ranson (3), believed that interruption of the supraoptico-hypophyseal tracts causes the neurohypophysis to become functionally inactive and leads to a deficiency of the antidiuretic hormone which is formed in the posterior lobe. When the entire pituitary gland is destroyed, no permanent severe diabetes insipidus occurs. This led von Hann (15) to deduce that diabetes insipidus cannot occur unless functioning anterior lobe is present. White and Heimbecker (16), using dogs, first removed the posterior lobe, producing severe diabetes insipidus. After a few weeks they extirpated the anterior lobe in the same animals. The polyuria began to diminish in a few weeks. These authors concluded that polyuria would remain at its maximum only if some anterior lobe was present. They did not state the amount of anterior

lobe tissue necessary for a permanent severe polyuria and polydypsia. Certainly, from our findings, it appears that very little of the anterior lobe tissue is necessary. Only about one-sixth of the parenchyma of the anterior lobe remained and still it was sufficient to maintain a very severe and permanent diabetes insipidus. It is to be noted that the deficiency in anterior lobe tissue was sufficient to produce a severe dwarfism.

In regard to the subject of pituitary dwarfism little new information can be added (17–20). The classification of human dwarfism is not completely satisfactory at present, primarily because attempts at classification have emphasized the external features of the individual rather than the underlying morbid process. The typical pituitary dwarfism or infantilism was first described by Lorain as "an arrest of development which affects the mass of the individual rather than any special organ, causing nothing more than disability, delicacy and smallness of body and a genital dystrophy—the individual is merely an adult in small mould." Certainly, our case fits into this description. Pituitary dwarfism results from a decrease of the eosinophilic cells of the pituitary. The chief cause of such a cell decrease is pressure upon the cells with subsequent atrophy secondary to some tumor growth, or an idiopathic deficiency or an arrested development of the pituitary gland. Destructive lesions such as observed in our case with a secondary fibrosis of the pituitary have not been reported. Although an occasional clinical report mentions the presence of diabetes insipidus in this type of dwarfism, no autopsy records are available to indicate the nature of the causative lesion. The present case seems unique in its clinical picture with substantiation by actual pathological studies.

SUMMARY

A case of pituitary infantilism is reported occurring in an 18-year-old girl who, at autopsy, showed bilateral localized lesions in the hypothalamus limited almost entirely to the region of the supraoptic nuclei. The patient manifested a permanent severe diabetes insipidus in spite of the presence of only one-sixth of the parenchyma of the anterior lobe of the hypophysis. There was an almost complete disappearance of the cells of the paraventricular nuclei as well as complete fibrosis of the neurohypophysis.

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BENZEDRINE SULFATE IN OBESITY

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FOR THE past few years benzedrine sulfate (amphetamine sulfate) has been widely used in various branches of medicine. Among the side-effects attributed to it, loss of weight has been frequently mentioned. This loss may be undesirable, particularly in mental patients already underweight (1–4), but it is usually followed by a rapid gain when therapy is discontinued. Moreover, loss is most frequent and most pronounced in obese patients (4–7).

Lesses and Myerson (8) reported favorably on the clinical use of benzedrine sulfate as an aid in weight reduction. We have been using the drug for the same purpose at Bellevue Hospital[1] since May, 1937. We give here the results of our observations.

Twelve of the patients in our series were more than 50% overweight (some even more than 100%), and there were 5 between 40% and 50%.

We first administered benzedrine sulfate to 13 obese female patients who had already been under our care for periods ranging from 4 to 71 weeks, with an average of 31.5 weeks. Treatment had consisted of restriction of diet to 600 to 1000 calories, and thyroid extract in appropriate cases. Most of these patients had responded well at the beginning of treatment, but had practically ceased to lose. For this reason, we tried benzedrine sulfate, which was given in daily doses ranging from 10 to 30 mg., and taken in 1 to 3 doses. Thyroid was discontinued in all cases, but the diet remained unaltered. It should be pointed out, however, that since all cases were ambulatory clinic patients, most of them on relief, it was impossible to control their diet with any degree of accuracy. For purposes of comparison we assume that variations in diet before and after the beginning of benzedrine therapy counterbalanced one another, unless of course the medication had a specific effect in determining food intake.

Table 1 shows the average weekly loss before and after benzedrine sulfate was given. Equal periods were taken where possible, but when the original period was shorter than the second, the average of the first is based on the entire pre-benzedrine period. Negative figures represent a gain of weight. The cases are arranged according to the amount of weight lost on benzedrine sulfate.

The table shows that the average loss after benzedrine sulfate was given was more than 7 times as rapid as before. The greatest weekly average without its aid (0.6 lb.) is smaller than the general average of the benzedrine period and is based on treatment of only the 3 original weeks. There was only one complete failure with benzedrine sulfate (*case 11*), whereas there were 7 with the original treatment (*case 9, 23, 16, 19, 15, 17, 27*); and *case 16* might well be called a failure until benzedrine therapy was instituted.

Since the loss on any treatment was greatest in nearly all cases during the first few weeks, it seemed interesting to compare the results with and without benzedrine in the original respective periods. For this comparison we were able to add an additional case (*14*) to those which appear in table 1. As before, the cases are grouped according to the amount of weight lost while on benzedrine.

[1] In the Endocrine Clinic and the Fourth Medical Division, Dr. Charles H. Hammach, Director.

TABLE 1

Case No.	Before benzedrine sulfate		On benzedrine sulfate	
	No. weeks	Ave. weekly loss, lb.	No. weeks	Ave. weekly loss, lb.
11	22	0.5	22	−0.19
9	4	0	43	0.19
23	12	−0.41	12	0.41
16	20	−0.05	20	0.6
4	4	0.5	5	0.6
24	26	0.07	26	0.65
20	32	0.25	32	0.65
19	13	−0.16	19	0.74
18	30	0.5	30	0.8
5	8	0.37	8	1.0
15	20	−0.3	20	1.2
17	4	0	15	1.53
27	4	0	5	2.4
Average	15.2	0.11	19.8	0.81

The first readings of this table are taken from the records at the beginning of all treatment, when the most marked response can be expected from the patient, whereas there was no interruption of therapy before the second readings. In spite of this, it is apparent that the response to the first 4 weeks of benzedrine sulfate medication, nearly always in conjunction with a low calorie diet, was more than twice as pronounced as the response to diet with or without thyroid in the first 4 weeks of all treatment.

Five cases were given benzedrine sulfate in conjunction with a low calorie diet from the beginning of therapy. The results appear in table 3.

Case 29 was the most dramatic in our series. He was a gigantic negro weighing 444 lb. On a diet of 2000 calories, with benzedrine sulfate, he lost 21 lb. in the first 2 weeks, and, as is shown in table 3, 43 lb. in the first 10 weeks.

The above results were noted in order to test the efficacy of benzedrine sulfate as an aid in adhering to dietary regime. In order to test whether it has any specific action of inducing loss of excess weight, we gave it to 5 patients, for at least a part of the period of treatment, without any restriction of diet. Two of these (*case 14, 15*)

TABLE 2

Case No.	Ave. weekly loss, lb., in first 4 weeks without benzedrine sulfate	Ave. weekly loss, lb., in first 4 weeks with benzedrine sulfate
16	0.5	0.2
20	1.5	0.5
4	0.5	0.75
11	2.5	1.0
23	1.25	1.0
19	0	1.25
5	0.25	1.25
24	−0.75	1.5
9	0	1.75
14	0.5	2.25
18	1.25	2.5
27	0.75	2.5
17	0	2.5
15	1.25	2.75
Average	0.68	1.55

TABLE 3

Case No.	No. weeks	Ave. weekly loss, lb., on benzedrine sulfate
21	13	0.7
25	38	0.8
26	7	1.3
28	11	1.9
29	10	4.3

also appear in table 2, and one (*case 15*) in table 1, but the period of treatment is necessarily different in both instances.

In 5 cases we compared the effects of alternate administration and withdrawal of the drug. In some instances the patient had voluntarily discontinued medication and

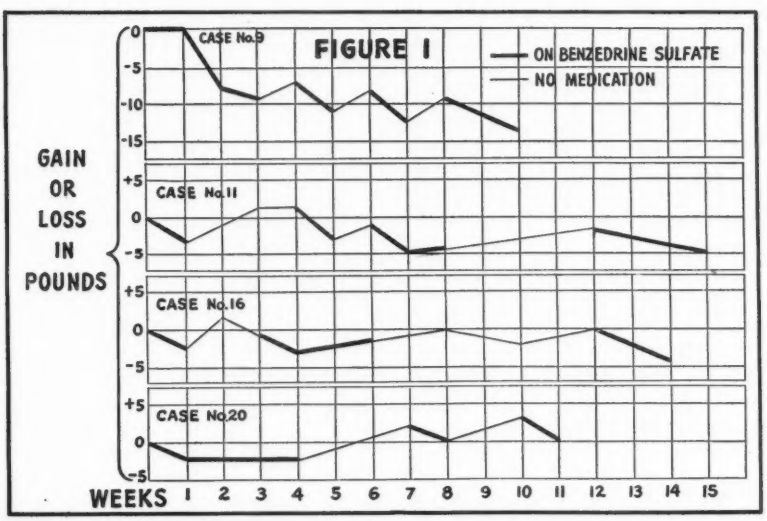
TABLE 4

Case No.	No. weeks	Ave. weekly loss, lb., on benzedrine sulfate
13	9	0.7
21	13	0.7
14	16	1.2
15	15	1.6
28	11	1.9

in others we expressly ordered discontinuance for purposes of control. The results of this comparison are plotted in figure 1. The heavy lines indicate the periods of medication.

It can readily be seen that one or two weeks without medication were usually sufficient, or more than sufficient, to counteract the effect of the weight loss effected with the aid of benzedrine sulfate.

Many of our patients stated spontaneously, or on being questioned, that they had lost their craving for food. Others saw no effect on appetite and some even thought appetite was increased by benzedrine sulfate. This latter effect was probably



subjective in most cases, though not invariably. Many patients found that they became more active while receiving the drug, because of a feeling of increased energy.

In order to get some information on the possibility of a specific action of benzedrine sulfate on body weight, we tried to eliminate in one case, insofar as possible, the factors of increased activity and decreased food intake. The subject of the experiment was an otherwise healthy 20-year-old girl of very short stature, weighing 304 lb. Before coming under observation she had worked in the kitchen. She was instructed

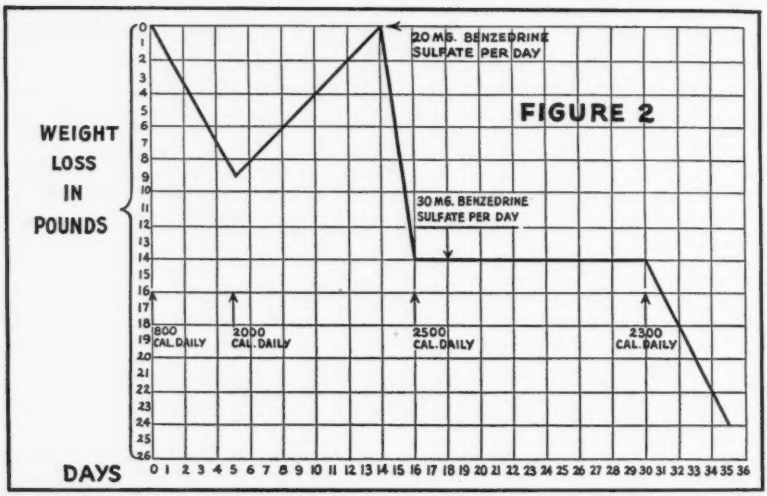


FIGURE 2

to stay in bed, but in practice spent about half the day lounging about the ward. In this way her activity was very considerably diminished.

In the first 5 days of the experiment she was put on a diet of 800 calories and lost 9 lb. during that time. She was then given an unlimited diet averaging about 2000 calories, which caused her to regain her former weight in 9 days. At that time she was given 20 mg. of benzedrine sulfate per day and lost 14 lb. in two days. Her diet was then increased to an average of 2500 calories, whereupon her weight remained stationary for 2 weeks, although the daily dose of benzedrine sulfate was increased to 30 mg. Finally, when she decreased her food intake to about 2300 calories on the same medication she lost an additional 10 lb. in 5 days.

Unfortunately this patient was not very reliable and probably neglected to record some of the food ingested. We observed her indulging very freely in sweets and thickly buttered sandwiches and she often ate left-overs on the trays of other patients. The figures should therefore be taken as minimum. The results are represented in figure 2.

It seems apparent that 2000 calories were more than sufficient for the patient's daily needs, since she gained weight on that amount. Therefore, 2300 calories were unquestionably far in excess of her needs, but in spite of this abnormal intake she lost rapidly while taking benzedrine sulfate.

In the entire series of cases, no untoward effects ascribable to benzedrine sulfate were observed except for occasional gastric upset when the drug was taken on an empty stomach. We found no increase of basal metabolic rate.

DISCUSSION

The results indicate clearly that benzedrine sulfate is a definite aid in weight

reduction. Tables 1 and 2 show that weight loss on benzedrine and diet is far greater than on diet with or without thyroid. Table 4 and figure 2 demonstrate that it often accomplishes the desired loss even without dietary restriction. A few considerations may throw some light on the mode of action by which this weight reduction is effected. Weight may be lost by the following means.

Decreased food intake. Obviously, when the calories ingested are decreased below the basal heat production the patient must lose weight. The will power necessary for such dietary restriction seems to be beyond the capacity of the average obese individual because of the mental and physical distress involved. Newburgh (21) stated that obesity might be called a disease of the appetite.

Myerson (8) has described the syndrome of "anhedonia" which is characterized by impaired mental and emotional satisfactions. Anhedonic patients may seek to compensate this subjective disorder through facile physical experiences, e.g. continual nibbling. Benzedrine sulfate was recommended as a dietary aid for such patients because it may have a beneficial effect on mood, as has often been stated in the literature. According to our observations, this effect of the drug has doubtless helped certain patients to adhere to the prescribed diet.

Decreased appetite due to benzedrine sulfate can also be attributed to decreased gastric tone (with the consequent delay in gastric emptying time usually reported), and decreased peristalsis, which are frequent findings (9–13). This smooth muscle effect may well help to rid the patient of the habit of eating between meals by delaying the recurrence of hunger.

MacLagan (14) studied the effect of various drugs on the appetite of rabbits and reported that ephedrine diminished food intake whereas benzedrine did not. However, his dosage of ephedrine was 500 mg., which should be enough to diminish appetite through its toxic effect, and benzedrine sulfate was given in 1 mg. doses, which might conceivably be insufficient to produce any specific effect on appetite. Furthermore, there is greater correlation between ephedrine and benzedrine than between rabbits and man, so that any conclusions to be drawn from such a study would not necessarily apply to a clinical study of the effect of drugs in man.

Correction of endocrine imbalance. It has been our experience, as well as that of Newburgh (21) and others, that even typical endocrine cases (pituitary or thyroid deficiency) are entirely governable by diet. This does not mean, of course, that appropriate glandular medication should not be administered when a diagnosis of such deficiency has been made. Thyroid extract is the standard therapy in cases of low basal metabolism. It acts by stimulating oxidation and thus burning up deposited body fat. In the present series thyroid was used in appropriate cases before benzedrine therapy was begun. Benzedrine sulfate, however, was found to be more effective. But the newer drug does not appear directly to stimulate the oxidation of body material. When B.M.R. readings have been higher after its administration, the increase has generally been attributed to increased muscular activity (15–17). As stated above, we found no evidence that benzedrine sulfate increases basal metabolic rate.

If benzedrine sulfate has any effect on body weight by influencing endocrine function, it is more probable that such an influence should be sought in the adrenals. Since benzedrine is a sympathomimetic drug, it is not unlikely that it may stimulate the adrenal glands, at least in certain individuals. Although most authors have found no effect on blood sugar, foreign workers (18–20) have observed a temporary rise contemporary with the pressor response. Hypoglycemia developing within a few hours after meals often generates the desire for food. Should this condition be remedied by benzedrine sulfate, or by any stimulation of the adrenals, the desired weight loss might be indirectly accomplished by controlling irregular indulgence in food.

Augmented energy output. The stimulus of oxidation through increased physical exertion is notoriously ineffective in weight reduction since it simultaneously causes increased appetite, which must be inhibited, or the results are negligible. It is well known that benzedrine sulfate usually stimulates physical as well as mental activity, but this factor can hardly explain its specific action on excess body weight.

Elimination of water. Newburgh (21) discussed two phenomena of weight reduction which often puzzle the patient and sometimes the physician. When weight loss exceeds the amount due to fat consumption, the difference must be attributed to increased water excretion. And vice versa, when patients fail to lose in spite of a strict regime, we know that the tissues are retaining surplus water. The failure to lose is naturally discouraging to the patient, and when the water eventually leaves the body, sometimes with dramatic suddenness, there is an excessively rapid weight reduction detrimental to the patient since it does not allow the skin time to adjust itself to the new tissue contours.

These phenomena were not observed during benzedrine sulfate medication. Figure 2, as we have shown, represents a case losing immediately (not a sudden loss after a period of stationary weight) on a diet of 2000 to 2300 calories with benzedrine sulfate. Without medication 2000 calories were sufficient to cause a rapid gain. Since appetite and activity cannot explain these results, the explanation must lie in the water balance. Other cases, e.g. those in figure 1, point to the same conclusion. Unfortunately it was not possible to check on water balance in this study, but it seems probable that benzedrine sulfate, at least in the obese, acts as a diuretic and tends to prevent the tissues from retaining superfluous water. The gradual weight loss thus effected may be beneficial, as it is not fraught with the possibility of deleterious effects associated with sudden loss.

SUMMARY

The effect of benzedrine sulfate on weight reduction was investigated in a controlled study of a group of 22 obese patients. It was found that benzedrine sulfate was a useful adjuvant in the treatment of obesity and more effective than thyroid extract in inducing weight reduction.

Nothing definite can be stated as to the mechanism of its action, but in addition to the effect on activity, mood and appetite already noted in the literature, it is suggested that it may act as a diuretic, preventing the retention of excess water in the tissues.

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THE COMPARATIVE ACTION OF GRADED DOSES OF INTERNATIONAL STANDARD GONADOTROPIC SUBSTANCE (CHORIONIC GONADOTROPHIN) ON NORMAL INFANTILE MALE AND FEMALE RATS

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THE THIRD INTERNATIONAL CONFERENCE on the Standardization of Hormones held in Geneva in August, 1938, under the auspices of the Permanent Commission on Biological Standardization of the Health Organization of the League of Nations arranged for the establishment of an International Standard Gonadotropic Substance (chorionic gonadotrophin). This standard was a mixture of samples submitted by 6 manufacturers in different countries. It was made available on April 1, 1939, as 10 mg. tablets, each tablet containing approximately 100 international units.

At present there is no generally accepted assay method for gonadotropic substances. The lack of agreement among investigators has resulted in a multitude of different rat, mouse and rabbit unit standards based on methods involving variations in the maturity and sex of the animal and degree of glandular response as determined by changes in weight or through histological examination of the gland.

With the establishment of an International Standard Chorionic Gonadotropic Substance it is imperative that consideration be given to the acceptance of a uniform assay procedure for urine samples and commercial preparations of this hormonal substance. This study presents certain pertinent data which would serve as a basis for such a method.

Rowe, Simond and Nelson (1) found the development of gross signs of luteinization in the ovaries of 30-day-old female rats (26 days old at the beginning of the assay) a desirable endpoint for estimation of potency of APL gonadotropic substances. D'Amour and D'Amour (2) have presented a critical study of assay methods utilizing the organ weights of infantile, 21-day-old, male and female rats. They also studied the gross vaginal and ovarian responses. Hamburger and Pedersen-Bjergaard (3) have presented a standardization curve obtained from the results of two different laboratories for two types of gonadotropic substances, pregnancy urine extract and pregnant mare serum extract. Heller, Lauson and Sevringhaus (4) considered the uterine weight changes as the most satisfactory end-point for the assay of gonadotropic factors from rat pituitary. Fevold (5) described an assay method for unfractionated pituitary extracts which gives a rough estimation of the relative amounts of FSH and LH factors by the comparison of ovarian with seminal vesicle response.

The present study was undertaken to make a comparison, on a statistical basis, of endocrine response resulting from graded doses of the International Standard Gonadotropic Substance on the following changes: uterine weight, ovarian weight, seminal vesicle weight, gross signs of luteinization and vaginal estrus.

EXPERIMENTAL

In this study the conditions under which the International Standard Gonadotropic Substance was assayed were maintained as uniform and as constant as possible. Wistar strain albino rats were employed. The diet of all animals consisted of Purina Fox Chow supplemented by adequate amounts of raw milk and fresh water. The animals were kept in a room maintained at 78 to 80° F. at all times.

The females received, twice daily at 8 A.M. and 4 P.M. for 3 days, subcutaneous doses of the standard powder dissolved in 0.25 cc. of water and were killed 96 to 100 hours after the initial injection. Vaginal smears, made immediately before the animals were terminated, were considered positive if they consisted of only squamous epithelial cells or a mixture of large epithelial cells and squamous cells with an essential absence of leucocytes. It was generally necessary to open the vagina (by careful

TABLE 1. THE EFFECT OF THE INTERNATIONAL STANDARD GONADOTROPIC SUBSTANCE ON THE BODY AND GLAND WEIGHTS OF IMMATURE FEMALE RATS

Dose, I.U.	0.0	0.5	0.75	1.0	1.5	2.0	3.0	4.0	5.0	6.0	8.0	10.0
No. of animals	36	10	8	15	16	19	11	16	11	14	10	4
Initial mean body wt. (gm.)	39	43	41	39	38	40	36	39	40	39	40	40
Standard error[1]	0.3	1.0	0.5	0.4	0.3	0.4	0.5	0.6	0.7	0.7	0.7	0.9
Final mean body wt. (gm.)	49	60	55	49	47	51	49	53	54	50	53	55
Standard error[1]	0.8	1.5	0.8	0.6	0.8	0.5	0.9	0.8	0.8	0.7	0.8	1.0
% body weight change from control		70	40	0	−10	10	30	40	40	10	30	50
Mean ovarian wt. (mg.)	8.9	8.5	6.3	6.8	6.9	11.6	11.6	15.0	19.6	19.5	24.7	23.7
Standard deviation[1]	1.3	1.5	0.9	1.1	1.8	2.7	1.7	2.5	3.8	2.1	2.5	1.4
Standard error	0.2	0.5	0.3	0.3	0.4	0.6	0.5	0.6	0.9	0.6	0.8	0.7
Mean uterine wt. (mg.)	14.4	17.7	52	60	66	94	94	93	98	103	103	102
Standard deviation	2.2	2.4	13.5	9.9	14.8	14.0	11.4	12.8	10.6	19.2	8.6	3.7
Standard error	0.4	0.8	4.8	2.6	3.7	3.2	3.4	3.2	3.2	5.1	2.3	1.9
Mean thymus wt. (mg.)[2]	109	125	139	120	94	115	84	104	103	86	113	110
Standard error	3.2	2.6	8.6	4.0	5.0	3.8	4.2	5.4	7.7	4.8	7.5	9.8
% showing estrus	0	0	62	53	81	100	100	100	100	100	100	100
% showing luteinization	0	0	0	4	3	21	59	88	82	89	95	100

[1] See footnote 2 for calculation.
[2] A correction factor of 3.3 mg. per gm. significant variation from mean body weight of 39 gm. should be applied.

manipulation with a blunt probe) of those animals receiving less than 1.5 I.U. The ovaries were examined grossly for hemorrhagic follicles as a sign of luteinization; in the calculation of percentage luteinization, doubtful observations received a value of 0.5 (table 1).

The ovaries and uteri were dissected free from the fallopian tubes and surrounding tissue and, with the thymus, were fixed 24 to 48 hours in Bouin's fixative. They were then transferred to 70 to 80% alcohol, carefully blotted between sheets of toweling, the uteri being free from intrauterine fluid, and weighed on a chainomatic analytical balance.

The male rats received, twice daily for 5 days, subcutaneous doses of the standard powder dissolved in 0.15 cc. of water and were killed 124 to 128 hours after the initial injection. The seminal vesicles and thymus were separated from the surrounding tissue, fixed and weighed in the manner described above.

Solutions of the standard powder were freshly prepared every 40 to 48 hours[1]

[1] S. Gurin, C. Bachman and O. W. Wilson, *J. Biol. Chem. 128*: 525. 1939, have shown that the powder is stable in the dry state but loses much of its activity after 48 hours when kept in aqueous solutions even in the cold. We have observed that solutions of the international standard powder stored in a refrigerator at 40° F. lose from 25 to 50% of their original potency in 30 days.

and when not in use were stored in a refrigerator at 40° F. The dry tablets of the standard were stored in a vial over a drying agent below 0° C.

The total doses ranged from 0.5 to 10.0 I.U. At least 8 animals were used in establishing each point. Statistical analyses were made of the data utilizing the formulae outlined by Burn (6). A graph (fig. 1) based on the calculated standard error[2,3] of 9 determinations, shows the effect of graded doses of the international

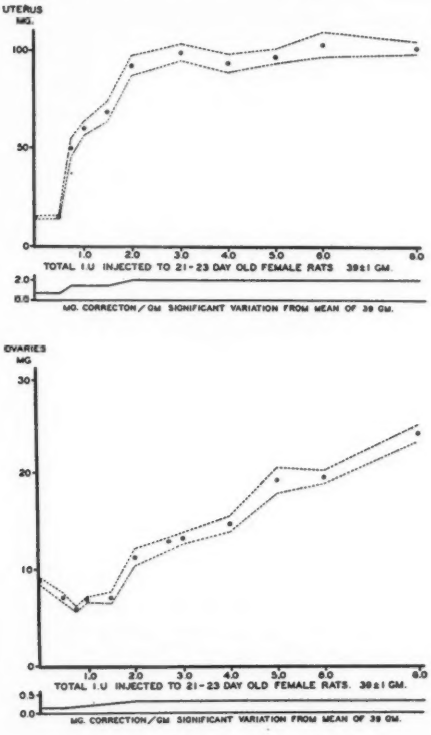

Fig. 1. Effect of graded doses of international standard gonadotropic substance on the ovarian and uterine weights of immature rats. (Based on the standard error of nine determinations.)

standard on the rat ovarian and uterine weights. A similar graph (fig. 2) shows the effect of graded doses of the international standard on the rat seminal vesicle weight. Below each curve is a graph for the correction of mean gland weights of groups of animals having a mean body weight significantly different from that upon which these graphs are based.

DISCUSSION

The mechanism of seminal vesicle response involves the testes. Most investigators believe that this response is due to the stimulation of the testes, by the gonadotropin,

[2] The standard deviation of a single observation (σ) is calculated from the formula $\sigma = \sqrt{\Sigma d^2/n-1}$. The standard deviation of the mean (standard error, ϵ) is obtained by dividing σ by $\sqrt{n}$.

[3] In this study if a single determination of d^2 was greater than $\frac{1}{2}\Sigma d^2$, the response was considered abnormal and ruled out. (This represented less than 1% of the observations.)

resulting in the formation of androgenic factors by the interstitial cells. The seminal vesicle responds to doses of the standard as low as 1.0 I.U. Its response to change in dose is most significant[4] from doses of 1.5 to 8.0 I.U. Through this range the curve approximates a straight line; a significant change in weight of the seminal vesicle occurs with a change in dosage of 0.8 I.U. Assays made in the range of 2 to 8 I.U. equivalents should therefore be accurate within 15 to 20%.

The initial ovarian weight decrease from doses of less than 1.5 I.U. was accompanied by a sharp rise in uterine weight. An analogous ovarian and uterine re-

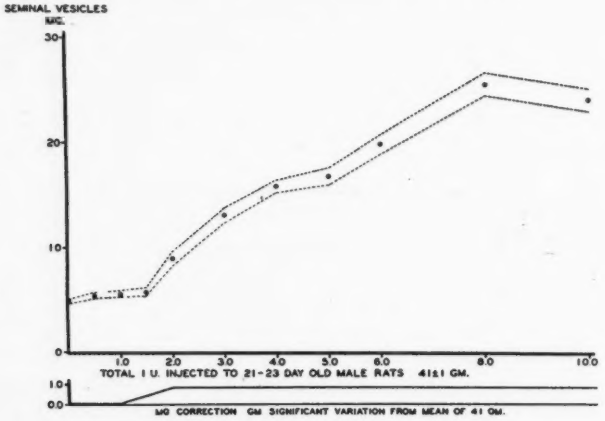

Fig. 2. EFFECT OF GRADED DOSES OF INTERNATIONAL STANDARD GONADOTROPIC SUBSTANCE ON THE SEMINAL VESICLE WEIGHT OF IMMATURE RATS. (Based on the standard error of 9 determinations.)

sponse to rat pituitary suspension was reported by Heller, Lauson and Sevringhaus (4) and Cartland and Nelson (7) reported a similar response to pregnant mare serum extract. Doses above 1.5 I.U. produced a steady rise in the weight of the ovary, the maximum effect being reached at doses near 8.0 I.U. At doses of 1.5 to 8.0 I.U. the curve again approximates a straight line with such a slope that a change in dose of 0.8 I.U. is significant.[4] Here again assays made in ranges of 2 to 8 I.U. equivalents should be accurate within 15 to 20%.

The mechanism of uterine enlargement obviously involves the ovary. The uterine weight change is capable of yielding quite sasisfactory results through the range of 0.5 to 2.0 I.U. The uterine response to small changes in dose is especially significant throughout that portion of the curve where a decrease in weight of the ovary was observed. The uterine weight change offers a very sensitive method of estimating the potency of gonadotropic extracts between 0.5 and 1.0 I.U., since significant[4] responses are observed to changes in doses of 0.1 I.U. Doses of 1.0 to 2.0 I.U. gave a good response to changes of 0.25 I.U. Above 2.0 I.U. the weight change was insignificant.

Butcher and Persike (8) reported that injection of chorionic gonadotropic substance (antuitrin-S) caused an arrested growth of the thymus in young albino rats. One observes a significant tendency for the mean thymus weight of the injected

[4] If the quotient $m_1 - m_2/\sqrt{\epsilon_1^2 + \epsilon_1^2}$ was greater than 2.0 a change in the mean was considered significant. (m_1 and m_2 are the two means and ϵ_1 and ϵ_2 are their respective standard errors.)

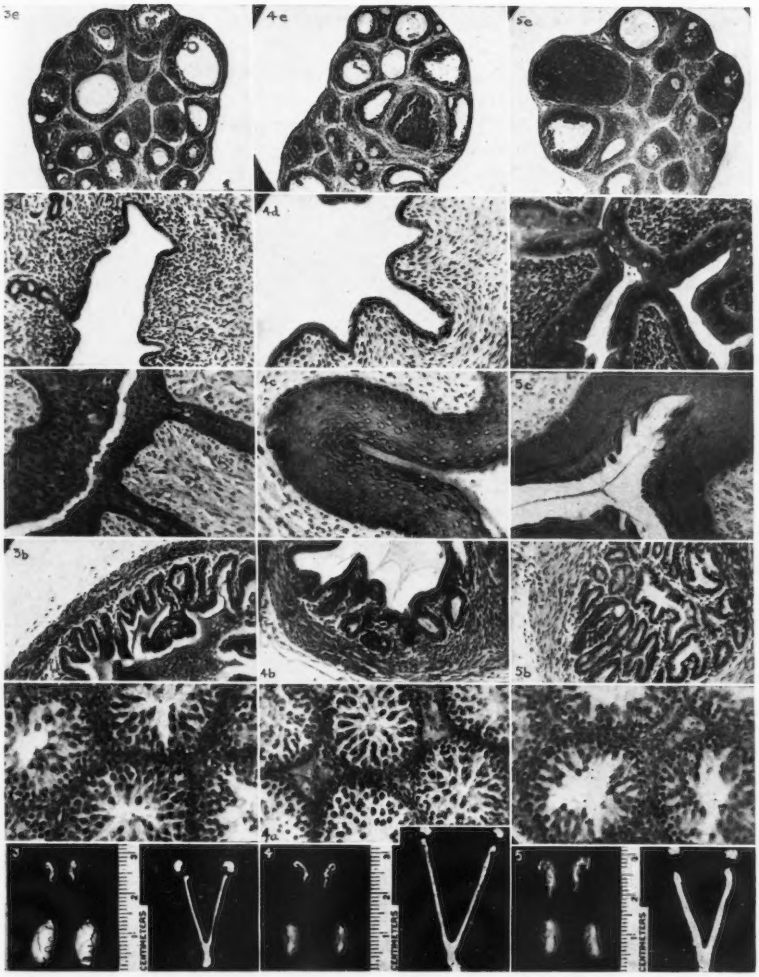

Fig. 3. Ovaries, uterus, seminal vesicles and testes of control infantile rats. Fig. 4. Same from female receiving 0.75 I.U. and male receiving 1.25 I.U. Fig. 5. Same from female receiving 8.0 I.U. and male receiving 10.0 I.U. Fig. 3 a, 4 a and 5 a, Testis from corresponding rats. Fig. 3 b, 4 b and 5 b, Seminal vesicle from corresponding rats. Fig. 3 c, 4 c and 5 c, Vagina from corresponding rats. Fig. 3 d, 4 d and 5 d, Uterus from corresponding rats. Fig. 3 e, 4 e and 5 e, Ovary from corresponding rats. All sections stained hematoxylin-eosin. Series a, b, c, and d ×270 and series e at ×60.

females to be less than the observed weight of the controls; this appears significant when doses of 3 to 6 I.U. are administered. In the male (possibly due to a longer injection period) there was a significant decrease of 10 to 20% in the mean thymus weight of animals receiving over 0.5 I.U. The mean thymus weights are recorded in tables 1 and 2.

No significant change in body weight was observed in the males during the injec-

tion period; however, the injected females generally showed a definite tendency to gain 10 to 40% more than their controls.

Gross examination of the ovaries and vagina and microscopic examination of vaginal smears made at the time the animal was killed showed that animals receiving 0.5 I.U. or less had closed vaginas. The vaginas of animals receiving 0.75 to 2.0 I.U. could frequently be opened by a probe while the vaginas of most animals receiving over 2.0 I.U. were open spontaneously at 96 to 100 hours.

TABLE 2. THE EFFECT OF THE INTERNATIONAL STANDARD GONADOTROFIC SUBSTANCE ON THE BODY AND GLAND WEIGHTS OF IMMATURE MALE RATS

Dose, I.U.	0.0	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	8.0	10.0
No. of animals	37	13	25	12	11	9	11	13	10	8	11
Initial mean body wt. (gm.)	41	45	38	42	41	36	39	41	41	36	42
Standard error[1]	0.4	0.9	0.5	0.7	0.7	0.4	0.8	0.5	0.5	1.2	0.5
Final mean body wt. (gm.)	58	65	52	56	63	55	57	61	57	66	60
Standard error	1.0	1.2	0.7	1.2	1.4	0.9	0.8	1.1	0.7	1.6	0.8
% body weight change from control		18	−18	−18	29	12	6	18	−6	18	0
Mean sem. vesc. wt. (mg.)	4.7	5.5	5.1	5.8	8.9	10.0	15.0	16.8	19.7	29.5	24.9
Standard deviation[1]	0.5	1.1	0.8	1.3	2.2	2.0	1.8	2.4	2.7	3.2	3.3
Standard error	0.1	0.3	0.2	0.4	0.7	0.7	0.6	0.7	0.8	1.1	1.0
Mean thymus wt. (mg.)[2]	125	129	100	102	127	93	100	124	115	136	102
Standard error	3.1	2.9	2.1	5.0	5.6	4.0	3.6	4.6	4.8	11.3	5.4

[1] See footnote 2 for calculation.

[2] A correction factor of 3.3 mg. per gm. significant variation from mean body weight of 39 gm. should be applied.

Table 1 shows the gross observation of changes occuring in the ovaries and vagina. The percentage of animals showing vaginal estrus was significant through the range of 0.5 to 2.0 I.U. The percentage of animals showing luteinization was significant through the range of 1.0 to 8.0 I.U.

Figures 3, 4 and 5 are photographs of the ovaries, uterus, seminal vesicles and testes illustrating gross changes observed at doses of 0.0, 0.75 and 8.0 I.U. respectively, in the female, and doses of 0.0, 1.25 and 10.0 I.U. in the male.

The lumen of the uterus in figure 4 was distended with fluid while that illustrated in figure 5 was compact and contained little fluid; the ovaries illustrated in figure 4 were grossly smaller than those shown in figure 3. Neither showed hemorrhagic follicles. The ovaries illustrated in figure 5 contained a number of hemorrhagic follicles.

Histological Changes

Practically all investigators agree that chorionic gonadotropic substances cause a marked increase in interstitial tissue of the testes of immature rats. Our findings in this respect were merely corroborative and were similar to those observed by other investigators, notably Rubinstein and Abarbanel (9).

Histologically, the testes of immature rats (fig. 3a) showed scant interstitial tissue and no mitotic figures were observed; the testes of immature treated animals receiving a total dose of 1.25 I.U. (fig. 4 a) apparently had a marked increase in interstitial tissue, moderate hyperemia and occasional mitotic figures; the testes of animals receiving 10.0 I.U. (fig. 5 a) showed interstitial tissue stimulation, marked hyperemia and frequent mitotic figures.

The seminal vesicles of control immature rats (fig. 3 b) had a non-secretory epithelium of cuboidal cells containing little cytoplasm and a thin muscular sheath; the epithelium was one cell in thickness. The seminal vesicle of the rat receiving 1.25 I.U. (fig. 4 b) had a somewhat heavier wall; the epithelial cells were somewhat taller and cytoplasm was forming in the supranuclear region; no granules were observed in the cytoplasm. The cells of the epithelium of the seminal vesicle of the rat receiving 10.0 I.U. (fig. 5 b) were tall and narrow and barely exceeded the nucleus in width. Conspicuous under high power magnification were granules of various sizes distributed in the cytoplasm of the supranuclear region. The lumen was quite proliferated and the muscular wall was considerably thickened. These changes have been described in detail as castration changes observed in normal males by Moore, Hughes and Gallagher (10).

The vaginal epithelium of the immature control rats (fig. 3 c) were composed of tightly packed non-secretory epithelial cells. The vaginal epithelium of a rat receiving 0.75 I.U. (fig. 4 c) was composed of a heavy layer of squamous epithelium with cornified cells at the surface. Rats receiving 8.0 I.U. (fig. 5 c) showed little change from figure 4 c.

The endometrium of the control rats (fig. 3 d) was composed of a single layer of cuboidal epithelial cells containing little cytoplasm. The uterine wall was quite thin. The endometrium of rats receiving 0.75 I.U. (fig. 4 d) was composed of a single layer of tall columnar epithelial cells containing considerable cytoplasm with the nucleus occupying the end distal to the lumen. The animals receiving 10.0 I.U. (fig. 5 d) had a thick proliferated secretory endometrium of irregular structure containing a large number of vacuolated cells; the myometrium was thickened.

The ovaries of the control rats (fig. 3 e) contained an occasional maturing follicle and numerous immature follicles with a large amount of interstitial tissue. The ovaries of rats receiving 0.75 I.U. (fig. 4 e) contained a number of maturing follicles but rarely showed luteinization with interstitial tissue considerably decreased. The ovaries of rats receiving 10.0 I.U. (fig. 5 e) generally contained a large number of maturing follicles and frequent corpora lutea.

DISCUSSION

Inasmuch as the International Standard Gonadotropic Substance is an unfractionated preparation it is probably composed of at least two active factors. Consequently one would expect the results obtained by the use of a single criterion of glandular response to give very little information with regard to the proportion of the different hormones in an extract. Therefore the comparative effects upon the ovarian, uterine and seminal vesicle response are of special significance.

SUMMARY

The comparative action of graded doses of International Standard Gonadotropic Substance (chorionic gonadotrophin) has been studied in 330 normal infantile male and female rats.

Curves have been presented showing the change in weight of the seminal vesicles, ovaries and uteri from doses of minimum effect to doses of maximum response.

Tables are presented showing the effects of graded doses of international standard on the following: body weight change during the injection period; mean ovarian, seminal vesicle, uterine and thymus weights and percentage of luteinization and vaginal cornification.

Using the ovarian, uterine or seminal vesicle weight changes the potency of solutions containing chorionic gonadotropic factors may be calculated from curves derived from these tables with an accuracy of 15 to 20% through a dosage range of 0.5 to 8.0 I.U. equivalents.

Comparison of ovarian and uterine responses to the chorionic standard revealed a striking similarity to the responses of the immature rat ovary and uterus to anterior pituitary and pregnant mare serum observed by others.

Histological changes induced in the ovaries, uterus, vagina, seminal vesicles and testes have been described and illustrated with photomicrographs.

The authors wish to express their appreciation to Frank Kalich for his assistance in preparation of histological specimens and the photography of illustrations. To Dr. Tom R. Hamilton we acknowledge counsel for cytological interpretation; however, the authors accept responsibility for statements and conclusions herein contained.

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THE RELATION OF THE ANTERIOR PITUITARY TO BILE PRODUCTION[1]

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FITZGERALD, LASZT, AND VERZÁR (1) recently emphasized that the selective action in intestinal absorption is influenced by hypophysectomy. They presented evidence indicating that the administration of the adrenocortical hormone increased the absorption rate of fat and glucose in the hypophysectomized rat. It was suggested that the A P influenced glucose absorption through the action of the adrenocorticotropic hormone upon the adrenal cortex. Althausen and Stockholm (2) and Russell (3) showed a direct relation between thyroxine and rate of glucose and oleic acid absorption.

Fat digestion and especially absorption is known to be regulated in no small part by the bile. Insoluble fatty acids combine with the bile acids to form soluble compounds whose diffusibility through the cells of the intestinal tract is much increased. The bile also stimulates peristalsis and lowers surface tension.

If the rate of bile production influenced the rate of fat absorption and further if fat absorption is influenced by hormones of the A P, it would seem reasonable to postulate that some A P factor might influence the rate of bile production. The reasonableness of such a theory is further indicated by the demonstration that A P extracts cause other changes in the liver such as the infiltration of fat (4, 5) and protein metabolism effect (6). An increase of bile secretion by the liver aiding in more rapid fat absorption from the intestine would thus be a further link in the process by which the A P influences and regulates fat metabolism.

EXPERIMENTAL

Procedure. Female guinea pigs were anesthetized by the use of avertin and ether. Bile was collected by canulating the duct of the gall bladder after tying off the intestinal bile duct, letting the bile run freely into a container for measurement. Bile was collected for a period of 1 hour immediately after the operation, during which time the animals remained under the anesthesia. The operations were usually performed between 7:00 and 9:00 P.M. although 2 preliminary tests conducted at 3:00 P.M. gave similar results. An initial A P extract prepared by Bergman and Turner (7), was injected 4, 6 and 8 hours before the beginning of bile collection.

Normal bile production. Seven guinea pigs weighing between 280 and 370 gm. were used to determine the normal rate of bile production (table 1). Two animals were injected with a protein extract of guinea pig muscle plus pure amino acids 6 hours before bile was collected. These animals showed an hourly rate of bile production varying from 2.5 to 3.2 cc. with an average of 2.94 cc. Only one animal was out-

[1] This study has been aided in part by a grant from the Committee on Research in Endocrinology of the National Research Council.

[2] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series, No. 642.

side the narrow range of 2.8 to 3.2 cc. The amount of bile produced the second hour was less than the first.

Observations. The injection of 25 mg. of A P initial extract into guinea pigs weighing from 300 to 414 gm. was followed by a definite increase in bile production measured 6 hours later (table 2). Even as early as 4 hours and as late as 8 hours there appeared to be production greater than the normal indicating that the effect continued for a considerable period. However, the yields at these times seemed to vary widely, in both periods production of over 7 cc. was obtained. This was a larger production than from any animal after a 6-hour period. The average production of bile from the 13 experimental animals was 5.45 cc. or an increase of about 85%.

TABLE 1. NORMAL PRODUCTION OF BILE IN THE FEMALE GUINEA PIG

Animal No.	Body weight	Treatment	Time	Bile production
	gm.		*hr.*	*cc./hr.*
1	280	—	—	3.20
2	333	—	—	2.50
3	310	—	—	2.90
4	325	—	—	2.80
5	338	—	—	3.10
1	322	50 mg. protein	6	3.10
2	370	50 mg. protein	6	2.95

TABLE 2. INFLUENCE OF AP EXTRACT ON BILE PRODUCTION

Animal No.	Body weight	Initial extract A P	Time from injection to collection	Bile production 1st hr.
	gm.	*mg.*	*hr.*	*cc.*
1	300	25	6	5.20
2	270	25	6	5.50
3	384	25	6	4.50
4	374	25	6	5.40
5	375	25	6	5.30
6	389	25	6	6.60
7	360	25	6	5.60
8	314	25	6	5.70
9	376	25	4	3.50
10	313	25	4	7.80
11	414	25	8	4.00
12	350	25	8	4.50
13	333	25	8	7.20

DISCUSSION

These data indicate that an extract of the A P plays another rôle in fat metabolism in addition to causing a fatty infiltration of the liver and a rapid decrease in the blood fat of the rabbit (8) and guinea pig (6). The definite increase in bile secretion is indirect evidence that the A P is also concerned in the rate of fat absorption from the intestine.

Kocour and Ivy (9) have demonstrated that the ingestion of raw meat and oils stimulate the output of bile after the first or second hour and increasing up to the sixth hour after feeding. Although Tanturi and Ivy (10) showed that bile production might be influenced by nervous stimulation, they tentatively concluded that an increased blood flow through the liver augmented bile output. It is quite possible that

the increased liver function after A P extract injection would increase blood flow which in turn would augment bile production.

In the normal animal it is probable that the ingestion of fat stimulates the discharge of the A P factor, either nervously or by chemical means, into the blood which then influences fat metabolism including the production of bile and the rapid absorption of fat.

SUMMARY

A study is reported upon the hourly rate of bile secretion of female guinea pigs weighing between 280 and 370 gm. taken at a definite time of the day under avertin and ether anesthesia. The average hourly production of 7 normal animals was 2.94 cc. with a range of 2.5 to 3.2 cc.

Following the injection of 25 mg. of an initial extract of the A P, there was a rise in bile secretion extending from 4 to 8 hours. The average hourly production of bile from 13 injected animals was 5.45 cc., an increase of about 85%.

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BIOLOGICAL INDICATORS OF ANDROGENIC ACTIVITY[1]

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IT IS GENERALLY recognized that birds are more satisfactory test animals than mammals for biological assays of androgens. However, the relative potency of various substances is known to differ when assayed on birds and on mammals. This fact, together with the greater difficulty of interpreting phenomena observed in birds as related to human problems, has served as an impetus for the improvement of the mammalian technics.

Androgenic activity in the mammal is determined by gross weight or histological examination of sexual accessories, or by functional methods. Our study is primarily concerned with a comparison of certain methods in which the weight of various accessory sex organs provides a measure of androgenic activity which can be obtained with relative ease. The histological status of the tissues is probably a more sensitive indicator (1), but that method involves delay as well as added labor and expense. Function, as determined by ejaculatory tests, is not suitable for quantitative discrimination in exploratory studies where there is a possibility that most of the animals will receive sufficient androgen to elicit this all-or-none response.

Considerable variation is found in the technics of various weight studies. Some experimenters report the weight of the vesicles (full or empty) and the prostate complex, while others select only a representative portion of the gland. It would be of interest to know which of these weight determinations is the most sensitive indicator of androgenic activity; and which is the least influenced by experimental errors.

The present study is limited to a comparison of the relative merits of the seminal vesicles full and empty, the ventral prostate, and a functional measure consisting of the weight of the vesicular fluid. These data were collected for another study (2).

PROCEDURE

Male rats weighing 150 ± 25 gm. were castrated and treated with androgens for 21 days. On the day following the last treatment the animals were killed. A dissecting microscope was used for the removal of the ventral prostate and seminal vesicles which were freed from all extrinsic tissue and weighed. The seminal vesicles were then pressed between paper towels and reweighed. Inspection of the opened vesicles showed little if any residual fluid after this method of expression.

The androgens used for the treatment were testosterone and testosterone propionate. Other variables in the procedures were the solvents employed, the dosage, and the routes of administration. The animals were divided into 12 groups including one of unoperated controls and one of untreated castrate controls.

A total of 219 animals was used in the previous report, but the weights of the

[1] This project supported in part by a grant from the Josiah Macy Jr. Foundation; and the materials furnished by Ciba Pharmaceutical Co.

empty vesicles were obtained for only 191 of them. The comparisons here reported are therefore based on these 191 animals. The average body weights, and organ weights are tabulated in full in the previous publication and will not be repeated here.

RESULTS

The validity of the various measures could be most accurately determined if all conditions had been constant except for a gradual increase in the amount of androgen administered. Lacking this objective criterion, validity was indirectly established by adding the weights of the vesicles full and empty, and the prostate, and thus deriving a composite value. This is a method frequently used in the absence of independent measures of a given function (3). Correlations were then made between this value and the weights of each of the organs. They were as follows: full seminal vesicles +0.97, empty seminal vesicles +0.96, prostate +0.92, and vesicular fluid +0.96. Intercorrelations between the organs were all over +0.86. These high correlations show that without doubt all of the indicators are measuring the same phenomena. However, the differences between the correlations of the various organs with the total cannot be taken as a difference in their respective validities because the factor of variability was not ruled out of the correlations.

It was considered possible that the ratio of the ventral prostate to the seminal vesicles might prove to be a more sensitive indicator of androgenic activity than the actual organ weights. However, the correlations between the ratios and the weights of the vesicles or prostate were practically zero, and so the method was discarded.

The sensitivity of the different indicators was determined by comparing their ability to demonstrate significant differences between the variously treated groups. For the most part all the organs weighed designated the same experimental groups as showing significant variations. However, the prostate responded to significantly smaller amounts of androgen than the seminal vesicles. This fact has previously been demonstrated by other workers (4).

The next question to be answered is how reliably, or consistently, do these weight determinations actually measure androgenic activity?

Degrees of reliability are most frequently computed by one of the correlation technics. In cases such as this the test-retest method is not appropriate because it is obviously impossible to repeat an experiment on animals which have been killed. The split-half method which was used serves the same purpose. The animals were aligned by groups in the order killed, and were divided into 2 main groups, the odd and even numbers. Correlations made between the organ weights of these 2 groups were as follows: empty vesicles, +0.91; full vesicles, +0.87; ventral prostates, +0.79; and vesicular fluid, +0.78. The differences between these correlations are not statistically significant.

Unless the data are converted into standard scores,[2] this method tends to exaggerate the correlations of the most variable tests, in this instance the weights of the full vesicles and the fluid. On the other hand, a method which penalizes a variable test, is the use of the standard error, or sigma, of a mean. This is often employed to indicate the degree of reliability of the measure (or test) by which the mean was derived. Since there is a two to one chance that if this experiment were repeated on other similar groups of animals the means would lie one sigma above or below the values actually obtained, the sigma is a good criterion of accuracy.

[2] The standard score is the difference between an individual measurement and the mean of a group divided by the sigma. This rules out the factor of variability.

The standard errors of the means of the various organ weights were as follows: empty vesicles, 7.54; ventral prostate, 8.09; vesicular fluid, 17.69; and full vesicles, 24.80.

There being no way of determining whether or not differences are significant when they are based on a single measure, the problem was approached from a slightly different angle. Instead of using the sigmas of the mean weights of 191 organs as given above, the sigmas of the means of each of the 12 experimental groups were averaged. These are shown in table 1, together with the differences between the averages. The critical ratios between the differences and their standard deviations are also shown (see footnote under table). Since a critical ratio of 3 or more is indicative of statistical significance, it may be seen that the weight of the empty vesicles

TABLE 1. THE RELIABILITY OF VARIOUS MEASURES OF ANDROGENIC ACTIVITY IN THE RAT

Measures	Averages of σ mean of 12 groups of animals	Differences between averages (C. R.[1] in parentheses)		
		Sem. ves. (full)	Sem. ves. (empty)	Vent. prostate
Sem. ves. (Full)	34.4			
Sem. ves. (Empty)	9.0	25.4 (4.5)		
Vent. prostate	13.5	20.9 (3.4)	4.5 (1.8)	
Vesicular fluid	29.2	5.2 (0.7)	20.2 (3.5)	15.7 (2.5)

[1] Critical Ratio $= \frac{\text{obtained difference between averages}}{\text{standard deviation of obtained diff.}}$.

is significantly less variable than that of the full vesicles and the fluid, but does not differ significantly from that of the ventral prostate; and that the weight of the ventral prostate is significantly more reliable than the weight of the full vesicles but not the weight of the fluid.

SUMMARY

A statistical analysis was made of the relative merits of the seminal vesicles full and empty, the ventral prostate, and the vesicular fluid as measures of androgenic activity in the rat. The organs of 191 rats were used in the study.

From the comparisons made we might conclude that if weight measurements are to be used in the assay of small amounts of androgen, the ventral prostate would be the most suitable organ for the purpose since it is the most sensitive indicator.

If the androgen activity is expected to be equal to or greater than the normal, the weight of the empty vesicles might be the best measure because of its slightly greater reliability. There is no apparent advantage in using more than one of these weight determinations in a single study.

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THE ACTION OF TRASENTIN ON THE RABBIT OVARY AND UTERUS[1]

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THE ANTISPASMODIC ACTION of trasentin[2] on the isolated intestine of the rabbit was first described by Meier (1), and a similar effect on the rabbit uterus was observed by Johnson and Reynolds (2). Preliminary studies carried out by us on the human uterine strip in vitro showed a loss of contractions without lowering of tonus.

Clinical studies by one of us (R. K.) revealed the following facts.

(*a*) Some cases of dysmenorrhea were relieved of their symptoms when 225 mg. of trasentin were given daily by mouth for 10 days preceeding the period, and during the course of the period.

(*b*) Numerous cases of so-called closed Fallopian tubes, as determined by transuterine insufflation (Rubin Test) were found to have patent tubes after the daily administration of 225 mg. of trasentin by mouth for the first 10 days of the cycle. All insufflations were done on the 10th or 11th day of the cycle.

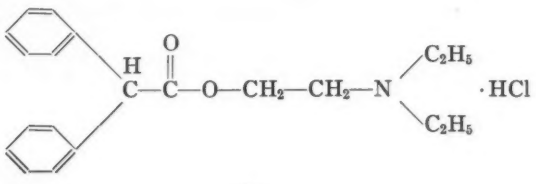
(*c*) Patients treated with trasentin frequently had their cycles, previously regular, shortened by one to six days.

(*d*) The incidence of pregnancy in a series of cases of functional sterility treated with trasentin increased from 20 to 28% (in the last 100 cases). This increase may not seem significant but it presented the greatest increase in a series covering 15 years.

The apparent therapeutic improvement in the sterile group and the frequent shortening of the menstrual cycle led us to investigate the action of trasentin on the ovary and uterus of the rabbit. In all 56 animals both mature and immature were used in the experiments. The mature rabbits had been kept isolated. The immature rabbits were of the type used for the routine Friedman test, and weighed from 1,000 to 1,500 gm. Trasentin was injected subcutaneously in 4 doses of 75 to 300 mg. Intraperitoneal injections caused marked dilatation and vascular engorgement of the

[1] Aided by a Grant from the Ciba Pharmaceutical Products, Inc.

[2] Trasentin is the hydrochloride of diphenylacetyl-diethylaminoethanol. (Ciba Pharmaceutical Products, Inc.)



intestines and loss of weight. The drug proved very toxic on intravenous injection, a fact previously noted by Johnson and Reynolds (2). The animals were killed 48, 72, or 96 hours after the first injection.

In the immature rabbits, after trasentin treatment, the ovaries showed a definite increase in the size and number of maturing follicles. There was no evidence of ovulation, except in one animal which showed a corpus luteum. Hemorrhages within follicles identical with those found in a positive Friedman test were frequently noted. The average weight of the ovaries was 112 mg., which is not significantly greater than in the normal controls.

The uteri were definitely enlarged, with an average weight of 612 mg. compared with 325 mg. for controls. The endometria were thickened and showed a typical estrogen reaction.

Three immature animals were injected with 50 mg. of trasentin daily for 3 days, and with 0.5 mg. of progesterone daily for the next 4 days. They were killed 24 hours after the last injection. The endometria of 2 of these animals showed a typical progestational phase; the third animal failed to respond because of illness resulting from the trasentin injections.[3] Immature rabbits injected with progesterone alone failed to show a pregestational endometrial phase.

When mature rabbits were used, the usual effect on the ovaries was the production of one or two hemorrhages in the follicles. Some ovaries showed a larger number of mature follicles, and in one animal (96 hours after the first injection) young corpora lutea were found. The ovarian weights were not significant. The uteri showed marked hyperemia and thickening and the endometria were typical of the "estrogen phase."

There was no effect on the uteri of castrated rabbits, either mature or immature.

The gonadotropic effect of trasentin could perhaps be explained by an action on the anterior pituitary by way of the sympathetic nervous system; studies on hypophysectomized rabbits are now in progress. In view of the therapeutic effects of trasentin in man we are now studying its effect on the excretion of follicle stimulating hormone in the urine, as well as its action on the human uterine strip in vitro and the intact uterus in situ. Derivatives of trasentin are also being studied. We have used the trasentin as supplied to us by the manufacturers without further attempts at recrystallization.

SUMMARY

Trasentin stimulated the development of follicles in the immature rabbit. In one animal ovulation occurred. It also produced in the uterus a typical estrogen phase which could then be converted into a progestational phase by crystalline progesterone. Marked enlargement of the uterus occurred.

The isolated mature rabbit reacted by a marked hyperemia of the uterus. The ovaries occasionally showed an increased number of mature follicles, and in one animal ovulation was produced. Both mature and immature rabbits showed hemorrhages into follicles.

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[3] Some animals did not tolerate trasentin in the doses given, and did not show the above reactions. Rats and mice almost invariably were killed by the trasentin. Doses as high as 1 gm. per day did not show the slightest toxic reaction in the human.

THE EFFECT OF ESTRIOL GLUCURONIDE ON THE SPONTANEOUS ACTIVITY OF SENILE MALE RATS

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As has recently been emphasized (1), the ageing organism presents a large variety of important and unsolved problems. That the terminal stages of life are commonly marked by gradually increasing decrepitude is a matter of common knowledge but the time, severity and speed of its development vary widely. Some men enjoy a fairly high degree of preservation of functions for as much as a century whereas others meet advancing decrepitude and ultimate death within three decades. An obvious need is to determine to what extent the decrepitude can be ameliorated or postponed. The fact that both longevity and senescent vigor are importantly determined by hereditary factors does not necessarily mean that they are not subject to considerable degrees of modification.

A study, of which this report constitutes one item, has been undertaken in an attempt to modify the vigor of ageing rats, using spontaneous activity as a criterion. To this end a variety of substances have been administered, including desiccated thyroid gland, various vitamin-rich materials, certain synthetic "hormones" and several endocrine glandular derivatives. For the most part no significant influence has been noted. The negative results with testosterone propionate (2) have been previously reported. The first preparation that gave any significant augmentation of spontaneous activity was a commercial product, emmenin, which is stated to consist essentially of a complex of estriol glucuronides. Subsequently other estrogens have been found to be effective. These will be reported later.

METHODS

The method of investigation has been to select from a group of ageing rats experimental subjects which were still apparently in good health but which showed relatively low levels of spontaneous activity. The activity was determined by means of automatically recording, revolving cages as described by Richter (3) and Hoskins (4) and their associates. In these cages the rats have the alternative of remaining quiescent in small housing compartments or of exercising in the revolving units. The method thus registers an algebraic summation of the effects of all the environmental and nutritional factors that influence neuro-muscular tension. The activity wheels that were used are 48 inches in circumference and the animals that were chosen usually made from 100 to 500 revolutions a week, most of them being in the lower range. The rats varied in age from 16 to 30 months. They had access to tap water and to Purina Dog Chow, ad libitum.

This report is based on 20 experiments made on 11 different animals, from 1 to 3 determinations having been secured on each. The estriol glucuronide was a fluid preparation and was mixed with a portion of the daily food. Commonly it was taken freely but an occasional animal displayed some reluctance in consuming it.[1]

[1] Our thanks are due to Ayerst, McKenna & Harrison for generous supplies of emmenin.

The dosage ranged from ½ to 5 cc. and the frequency of its administration from 3 to 7 times per week. The dosage most commonly used was 2 cc., 5 times a week. The duration of treatment was generally 3 or 5 weeks. In most instances the body weights of the individual rats were determined weekly. The data herein reported

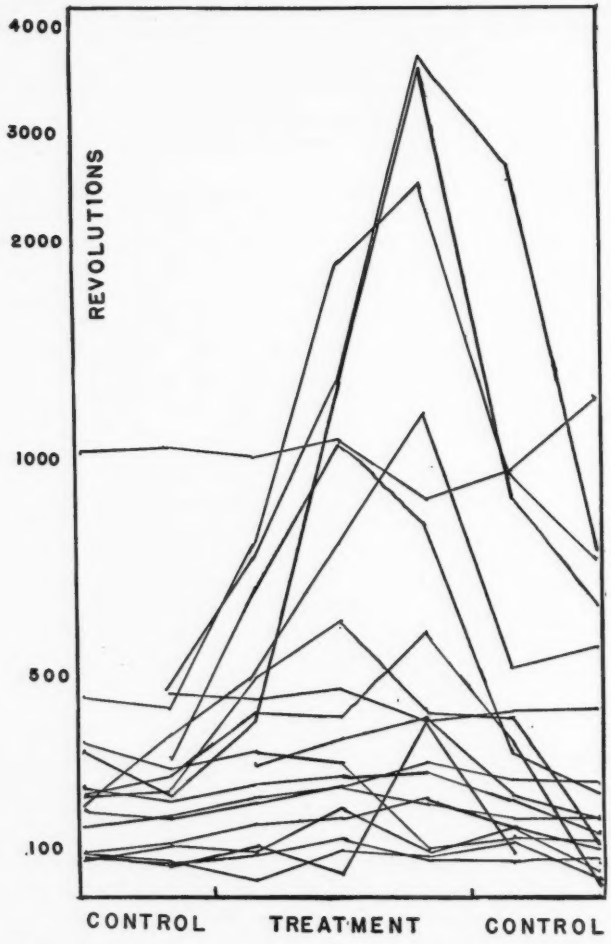

Fig. 1. Effect of estriol glucuronide upon spontaneous activity. Ordinates, revolutions per week of activity wheels. Note staggering of ordinate values. Control period, 2 weeks; treatment period, 3 weeks. Second control period, 2 weeks.

were unselected except that any rat dying during the course of the experiment or within one week after discontinuance of the treatment was excluded.

OBSERVATIONS

The results of the experiment as a whole are set forth in figure 1. The upward trend of the activity levels coincident with the administration of the estrogen is

unmistakable. It will be observed that in only 2 instances was there a decrease in activity. In general the reaction was greatest in those rats which showed the higher initial levels but that animal which had the highest level was one of the two which showed a depression during treatment. Had we been dealing merely with random irregularity, the depressions and augmentations should of course have been approximately equal in number and extent. Further evidence of the validity of the group trend was found in the repeatability of the phenomenon in individual cases. This was most strikingly seen in the case of the rat shown in figure 2 which was, incidentally,

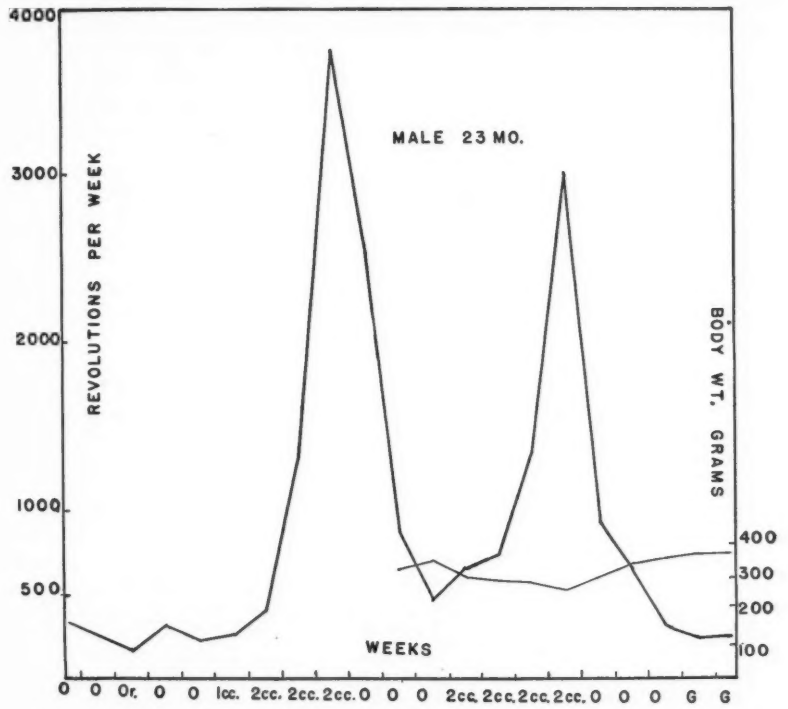

Fig. 2. Effect of estriol glucuronide in 2 tests in most reactive subject. Peaked curves, revolutions per week; flat curve, body weight. Symbols: O, no treatment; Or, testosterone propionate; 1 cc. or 2 cc., daily dosage of emmenin; G, gonadotropic preparation.

the most vigorous reactor of the group. Further evidence of validity lies in the fact that two other estrogen preparations, to be reported later, have given similar and indeed rather more consistent increases in activity.

In addition to the augmentation of spontaneous activity there was noted in all cases a diminution of body weight. The loss ranged from 23 to 91 gm. in the various instances. The weight loss may be ascribed in part to dehydration but in view of its magnitude it must have been due largely to genuine tissue depletion. No consistent correlation could be recognized between the weight loss and either dosage or degree of augmentation of activity though no doubt a larger series would permit the recognition of trends in these matters. In general the weight was regained within 1 to 3

weeks after the treatment was discontinued. Whether the rather sudden changes in weight had, as such, any deleterious or beneficial effect upon the organism is not evident from the data at hand. In 2 instances death occurred during or immediately following conclusion of the tests but in the age group that was studied 'spontaneous' death is not infrequent, hence it is not clear that the medication was a factor in the two instances noted.

DISCUSSION

A question of major interest is what effect the stimulating medication may have had upon longevity. The data at hand permit no final judgment on this point but the impression has been gained that the life span was not materially influenced. When ageing rats are kept continuously in activity cages, as was the case in these experiments, for periods of weeks or months there is noted a gradual decline in the activity level. The slope of this decline appeared not to have been significantly altered by the estrogen treatments. Much more extensive data would be required, however, finally to determine the matter.

Another important problem is the mechanism whereby the augmentation of activity was brought about. Had the rats been obese at the beginning of the tests one could ascribe the increase merely to the disposal of interfering fat tissue, but the animals were in merely an ordinary state of good nutrition. It may be noted that the study is still in an exploratory phase and opportunity has not yet been had to investigate this matter of mechanism. The need for such investigation is, of course, obvious.

SUMMARY

Twenty tests were made on 11 senescent or senile male rats of the effects of estriol glucuronide on spontaneous activity. The dosage ranged from ½ to 5 cc. of the commercial product, emmenin. It was administered from 3 to 7 times a week, for periods of 3 to 5 weeks. The most common regimen was 2 cc., 5 times weekly. The effect in general was an augmentation of activity. The effect was proved to be repeatable in individual cases. The underlying cause of the augmentation has not been determined.

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INHIBITION OF PELVIC CHANGES OCCURRING DURING PREGNANCY IN MICE BY TESTOSTERONE PROPIONATE[1]

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DURING LATE PREGNANCY the pelvis of the guinea pig enlarges by a hypertrophy of an interpubic ligament. These changes have been reproduced experimentally in female or male guinea pigs following adequate treatment with estrogens and relaxin (1). They involved a transformation of the interpubic synchondrosis to a pubic symphysis and then, associated with some bone destruction, a proliferation of the fibrous ligament to separate the pubes for variable distances (2, 3). A sexual dimorphism of the pelvis also exists in the pocket gopher, first becoming apparent at sexual maturity. The pubic bones were decalcified at the symphysis under the influence of the ovarian hormone (4). A pelvic sexual dimorphism also occurs in another rodent, the mouse (5). The pubic bones separated at the symphysis during the first pregnancy and these changes were augmented with succeeding pregnancies. The alterations of pelvic structure were reproduced and augmented by the injections of estrogens. Male mice receiving estrogens not only showed a development of an interpubic ligament but an extensive decalcification of the pubes. At the same time new osseous tissue replaced the marrow in other bones (6) increasing the ash content and altering the calcium phosphorus ratio (7).

The morphological and chemical changes in the bones of mice given estrogens were prevented if testosterone propionate was given simultaneously (7, 8). This might indicate that in untreated mice the sex changes are either positively induced as in females or inhibited as might occur in males by the action of androgens.

Relaxation of the pubic symphysis occasionally occurs during pregnancy in women (9). It was, of course, unknown whether or not these changes were associated with abnormal endocrine factors. The present experiments were conducted to determine the influence of testosterone propionate on the normally occurring pelvic changes associated with pregnancy in mice.

MATERIALS AND METHODS

Two series of experiments were undertaken. In both hybrid mice were used. Twenty mice were used in the first series. The onset of pregnancy was determined by the detection of vaginal plugs or sperm in the vaginal smears. Ten mice were injected with testosterone propionate and in 6 of these pregnancy continued for 19 days or more (table 1); the other 4 mice either did not become pregnant or pregnancy was interrupted in the early stages. Injections of testosterone propionate[3] (1.25 mg. every

[1] This investigation has been supported in part by grants from the Anna Fuller Fund, the Jane Coffin Childs Memorial Fund for Medical Research, Fluid Research Fund of Yale University School of Medicine and a special grant from the Belgian-American Educational Foundation.

[2] Belgian American Educational Foundation Fellow (1938–39) now at Department of Anatomy, Columbia University.

[3] The testosterone propionate (oreton) was generously supplied by Dr. Max Gilbert of the Schering Corporation, Bloomfield, N. J.

other day) were started at from the eleventh to the thirteenth day after mating. Of the 10 untreated mice 6 gave birth to normal litters at 20 to 22 days after the first mating. The other 4 mice in this series became pregnant following a 2nd mating. In the second series of 16 mice the injections of testosterone propionate (1.25 mg. daily) were started in 8 animals at the time of detection of the placental sign. The 8 other mice of the same litters were mated and allowed to continue pregnancy without injection of androgens.

The mice were killed if they successfully delivered, or observed during prolonged parturition. The length of the interpubic ligaments was measured as the shortest distance between the pubic rami.

TABLE 1. PELVIC CHANGES IN MICE RECEIVING TESTOSTERONE PROPIONATE (1.25 MG. EVERY SECOND DAY) AND IN UNTREATED CONTROLS. STAGE OF PREGNANCY WAS DETERMINED FROM DAY OF MATING.

No. of mice	Day pregnancy started	Total testosterone propionate	Day removed	Length of interpubic ligament
		mg.		*mm.*
1	13	5.0	22nd day 1 day postpartum	<1
1	11	7.5	23rd day Prolonged parturition	<1
1	11	6.25	20th day Died in parturition	1
1	11	4.5	Aborted 19 days	0.5
1	12	6.25	20th day Died in parturition	<1
1	12	5.0	20th day 1 day postpartum	<1
		Untreated		
10			20–22	2.5–4.–

RESULTS

The observations on the first series of animals are shown in table 1 and in the second series in table 2. In the first series a dead litter was delivered on the 19th day after copulation. One mouse was killed on the 20th day, one day after delivering a living litter. Two mice died during parturition on the 20th day and another died after a prolonged 2-day parturition on the 23rd day after mating. The 6th female was killed one day post-partum and on the 22nd day after mating. The 2 mice dying during delivery and the one mouse killed after the second day of a prolonged parturition had macerated fetuses in the cervical canals and upper vaginas. These 3 mice had interpubic ligaments of 1 mm. or less in length. Interpubic ligaments of similar lengths were found in the other 2 mice giving birth to normal litters and the one aborting on the 19th day. The day of pregnancy on which injections started, within the range studied, did not alter the response. The 10 untreated pregnant mice had interpubic ligaments of 2.5 to 4 mm. Four of these mice had been pseudopregnant once previously.

In the 2nd series the existence of pregnancy was determined by the placental sign. Of 8 females given 1.25 mg. of testosterone propionate daily for 8 to 11 days,

4 gave birth to dead young and 4 to living litters. The interpublic ligaments were 1 to 1.5 mm. in the former group and 0.5 mm. to 2 mm. in the second group. Two of the mice giving birth to dead young had macerated fetuses in the cervix at the time of autopsy.

The average length of the interpubic ligaments in 8 non-treated control mice at the day of parturition was 3.4 mm. (2 to 5 mm.). An average of 5.6 young were born per litter.

TABLE 2. PELVIC CHANGES IN MICE RECEIVING TESTOSTERONE PROPIONATE (1.25 MG. DAILY) AND IN UNTREATED CONTROLS. INJECTIONS STARTED ON DAY THE PLACENTAL SIGN WAS OBSERVED.

No. of mice	No. of days injected	Total dose	Length of interpubic ligament	Size and condition of litter
		mg.	*mm.*	
1	11	13.75	1.5	5 young, all dead
1	8	10.0	1.0	5 young, all dead
1	10	12.5	1.0	3 young, all dead
1	10	12.5	1.0	7 young, all dead
1	10	12.5	2.0	6 young, all alive
1[1]	8	10.0	1.5	7 young, all alive
1	10	12.5	1.0	5 young, all alive
1	11	13.75	0.5	6 young, all alive
Averages:			1.2	5.4 young per litter
		Untreated		
8			2–5 (3.4 average)	

[1] This mouse was apparently more than 11 days pregnant when blood first appeared in the vagina.

When testosterone was given in smaller doses to other mice of this series, 1.25 mg. every other day from the day when blood appeared in the vagina (placental sign) till parturition, the effect was less constant. In 4 mice interpubic ligaments of 3 to 4 mm. were found and in 4 others the ligaments measured 0.75 mm. to 1.75 mm. All gave birth to living young.

DISCUSSION

The influence of pregnancy on the formation of a ligamentous separation of the pubes of the mouse (5) was progressive during the first few pregnancies. Interpubic ligaments of 2 to 2.5 mm. were observed in primiparous mice and longer ligaments in mice that had several litters. Other experiments (10, 11) showed that longer ligaments were present in primipara immediately following delivery. These observations indicate that the interpubic ligaments may shorten somewhat following parturition as the shorter pubic separations were found in mice several months postpartum. The average length of the interpubic ligaments of the primiparous mice in the present study was 3.5 mm. The interpubic ligaments developed largely during the last 8 to 10 days of pregnancy in primipara (10).

Weekly injections of from 100 to 500 I.U. of estradiol benzoate induced similar separation of the pubes and also a partial resorption of the pubic rami. These changes occurred more slowly in males than in females. The influence of 33.3γ of estradiol benzoate injected weekly was entirely inhibited by the simultaneous injection of 1.25 mg. of testosterone benzoate weekly (8). The present experiments demonstrated that the injection of testosterone propionate inhibited the separation of the interpubic ligaments during normal pregnancy and indicated that in this species the inhibition of the separation of the pubes might be the factor in the impairment of the birth mechanism.

The rapid development of the interpubic ligament as occurs during the last half of the first pregnancy has never been reproduced experimentally. The fact that such large amounts of testosterone propionate are required to inhibit this separation during normal pregnancy (amounts that, injected weekly, will inhibit the action of 33.3γ of estradiol benzoate injected weekly in non-pregnant mice) indicated either that some accessory element was present during normal pregnancy or that estrogen occurred in highly effective amounts during pregnancy. The incomplete inhibition of interpubic ligament formation by testosterone during normal pregnancy might be the result of the comparatively late stages at which the injections were started.

Though it was impossible to quantitate the changes occurring in the ileo-sacral ligaments in as definite a manner as at the pubic symphysis it was indicated that some relaxation occurred and likewise that it was prevented or partially inhibited by testosterone.

Whether or not the same endocrine factors will act similarly in other species which show regularly a sexual dimorphism of the pelves or in women in whom such changes occasionally occur is unknown. It must be borne in mind, however, that testosterone propionate when administered at certain times during pregnancy will alter sex differentiation of the female fetus.

SUMMARY

Testosterone propionate was injected subcutaneously in mice at the rate of 1.25 mg. daily or every other day from the 11th to 13th day or from the detection of the placental sign to the end of pregnancy. The interpubic ligaments of those mice receiving the larger doses or smaller doses for longer periods were largely but not completely inhibited. Some mice died with macerated fetuses in the cervix and upper vagina and 4 mice gave birth to macerated fetuses. Other mice with similar restricted pelvic changes gave birth to living young. Large amounts of androgen were requured to inhibit the pelvic changes during pregnancy.

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THE EFFECT OF TESTOSTERONE PROPIONATE ON THE BLACK-CROWNED NIGHT HERON[1,2]

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DURING A STUDY of the social behavior of the night heron, *Nycticorax nycticorax hoactli* (Gmelin), previously reported (1), it was discovered that both sexes of this species undergo a marked increase in the pigmentation of the lores, lower mandible and buccal cavity, also a conspicuous vascularization of the legs, during the breeding season. When both sexes of a species of bird undergo the same seasonal development of a secondary sexual character it has been assumed, as in the case of *Larus ridibundus*, that the male and female sex hormones were producing identical effects (2). The recent experiments of Witschi and Miller (3) on the starling, *Sturnus vulgaris*, however, indicate that a male hormone may be present in sufficient amounts in both sexes during the breeding season to be responsible for these secondary sexual characters. This discovery raises the question as to why the females of those species, which owe their secondary sexual characters to male hormone, do not develop male behavior.

Little is known in regard to the breeding behavior of the starling under laboratory conditions but black-crowned night herons, free in a large, well-lighted room, retain the same patterns of social behavior they exhibit in the field. These herons, therefore, seem well suited for studying the rôle of hormones in regulating their social behavior.

MATERIAL AND PROCEDURE

Three sets of herons were employed in this study. The first were chicks approximately 20 days of age, collected in the nest or from the nest vicinity. The second were sexually mature birds which still retained their juvenile brown plumage, wholly or in part. Most of these had been reared from the chick stage in the laboratory. The third were adults with the full white and greenish-black plumage of the mature bird. A number of these had been collected in the field as mature birds but the majority had been reared in the laboratory from an immature stage. The black-crowned night heron undergoes a sequence of changes in the coloration of its plumage during the first 2 years of its life but, as none of its color changes are influenced by sex hormones, the species must be grouped with the pigeon, guinea fowl, Brewer's blackbird, South American plover, English sparrow and starling (4, 3) as a form with plumage color free of gonadal control. The iris of both sexes of the night heron also changes from a yellow or orange to a red during ontogeny, the latter color developing at approximately the same time as the mature plumage. Iris color, like the plumage of the night heron, undergoes no changes during the breeding season.

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In striking contrast, the color of the bill, lores and legs changes markedly during the breeding season. The lower mandible, lores and legs of the immature are greenish-yellow. Adults, out of season, have a greenish- or bluish-white lore, pale greenish or buff-colored legs. The upper mandible is a blackish-brown, the lower mandible lighter, especially caudally. The buccal cavity is a pale green or pinkish, frequently blotched with grey. With the approach of the breeding season the lores become purplish and then bluish-black, both upper and lower mandible change to a lustrous black and the legs assume a rich pink tone. These colors fade soon after the eggs hatch but are assumed again at the beginning of a second courtship, which may occur in the same spring as the first under laboratory conditions.

The birds were sexed by a direct examination of the gonads through an incision made between the last two ribs. In one series of 10 birds, the gonads were left intact after measuring them with dividers. In another series of 10 birds, the gonads were removed with an electrocautery.

As shown in tables 1 to 3, both the gonadectomized and the control series were injected with a series of sex hormones. The estrogens were: estrone acetate, theelin (Parke, Davis), follutein and amniotin (Squibb). The androgen was perandren (testosterone propionate, Ciba). A pituitary extract, whole sheep gland (Parke, Davis), was also employed. A control series of birds was maintained in the same type of cage as the experimental series.

RESULTS

Effect of sex hormones on the sexual pigmentation of the black-crowned night heron. As shown in table 1, testosterone propionate (perandren), injected into a 7-month-old

TABLE 1. EFFECT OF PITUITARY EXTRACTS, ESTROGENS AND ANDROGENS ON THE SEXUAL PIGMENTATION OF NORMAL BLACK-CROWNED NIGHT HERONS

Preparation	Sex and bird No.	Age	Duration of exp.	Dosage	Amt. injected	Gonad size		Remarks
						Start	End	
		mo.		cc.	cc.	mm.	mm.	
Whole sheep pituitary	♂ YP-O	4	9/1 –10/4	0.75	15	9X3	10X3	No change in pigmentation of lore, mouth or feet.
	♀ Y-B	4	9/1 –10/4	0.75	15	13X6	13X5	
Follutein	♀ RP-Y	4	9/16–10/20	0.3	6.9=690 R.U.	16X5	15X5	
	♀ RP-B	4	9/16–10/20	0.5	11.5=1150 R.U.	14X4	15X4	
Theelin	♂ YP-R	5	9/27–10/19	0.45 in H_2O	6.0 =16,200 I.U.	10X3	10X3	
			10/20–11/12	0.50 in oil	7.5	10X3	12.5X3	
	♀ YP-Y	5	9/27–10/19	0.45 in H_2O	6.0 =16,200 I.U.	15X5	16X5	
			10/20–11/12	0.50 in oil	7.5	16X5	16X8	
Amniotin	♀ YB-Y	6	10/13–11/12	0.25	5.0=50,000 I.U.	15X3	15X7	
	♀ BP-B	6	10/13–11/12	0.25	5.0=50,000 I.U.	13X4	15X5	
Estrone acetate	♀ 406		8/4 – 8/19	0.50	5.0=50,000 I.U.	11X?	14.5X6	
Perandren	♂ SYP-O	7	12/16–12/27	0.50	3.0=15,000 γ	8X2.5	8.5X3	After 2 inject., roof of mouth becoming pigmented. After 4 inject., lore black, mouth black, voice guttural, eyes popping.
	♀ SY-O	7	12/16–12/27	0.50	3.0=15,000 γ	14X4	14X6	After 2 inject., black in roof of mouth spreading extensively. After 4 inject., lore and eyelids are mixture of black and red. After 5 inject., lore blacker, voice gasping.

heron of either sex, quickly induced the development of black pigment in the lores and buccal cavity. A darkening of the lower mandible followed and the legs turned slightly pinkish. Night herons which breed during the first spring of their lives never

attain the rich pink tones of their legs found in the fully mature bird (1). The change induced by the testosterone propionate was equal to the maximum change in young birds.

Castration of herons before the breeding season invariably prevents the development of these changes in coloration of head and limbs. At the height of the breeding season, after 2 eggs had been deposited by pair *132-13*, both birds of this pair were gonadectomized. Ten to 14 days later both birds had lost their sexual colors of lores, buccal cavity and legs completely. Since ovariotomy is always difficult in the female, a post-mortem examination of *bird 13* was made 4 months later. This showed that gonadectomy had been complete.

As shown in table 2, the injection of testosterone propionate into gonadectomized

TABLE 2. EFFECT OF PITUITARY EXTRACTS, ESTROGENS AND ANDROGENS ON THE SEXUAL PIGMENTATION OF THE GONADECTOMIZED BLACK-CROWNED NIGHT HERON

Preparation	Sex and bird No.	Age	Duration of exp.	Dosage	Amt. injected	Remarks
				cc.	cc.	
Whole sheep pituitary	♀ *31*	1½ yr.	3/7–3/29	0.75	13.25	No breeding pigmentation developed.
	♂ R2	Adult	3/7–3/29	0.75	13.25	
Theelin	♀ *Y-GG*	10 mo.	3/7–3/29	0.50	9.5 =19,000 I.U.	
	♂ *311*	10 mo.	3/7–3/29	0.50	9.5 =19,000 I.U.	
Amniotin	♀ 333	10 mo.	3/7–3/29	0.25	4.75=47,500 I.U.	
	♂ 422	10 mo.	3/7–3/29	0.25	4.75=47,500 I.U.	
Perandren	♀ *123*	1½ yr.	2/7–2/18	0.25	2.75=13,750 γ	After 3 inject., pigmentation of feet at max. After 6 inject., voice becomes gasping. After 8 inject., complete breeding pigmentation
	♂ *113*	1½ yr.	2/7–2/18	0.25	2.75=13,750 γ	After 3 inject., typical breeding pigmentation. Red color in legs lagging behind that of ♀. After 5 inject., leg color at maximum. After 6 inject., voice guttural.
	♀ 322	10 mo.	3/7–3/29	0.25	4.25=21,250 γ	After 3 inject., eyelids and lore purple; black in roof of mouth, spreading from cleft forward. After 4 inject., legs becoming red. After 5 inject., tongue totally black. After 8 inject., voice guttural.
	♂ *411*	10 mo.	3/7–3/29	0.25	4.25=21,250 γ	After 3 inject., eyelids and lore purple. After 5 inject., breeding pigmentation almost complete. After 6 inject., voice guttural.
Control	♀ *112*	1½ yr.	2/7–2/18	no treatment		No change in pigmentation.
	♂ *121*	1½ yr.	2/7–2/18	no treatment		

birds of either sex will induce the development of the complete change of color in bill, buccal cavity and legs. Injections of 1.25 mg. of testosterone propionate were made into the pectoral muscles of the birds, 17 of these injections being given during a period of 3 weeks. The first change was found in the lores which became purplish before turning bluish-black (fig. 1, 2).

As the injections continued, the legs became pink and the roof, floor of the mouth, and tongue began to blacken. A similar melanization began at the base of the bill and the light areas in the lower mandible became obscured. The darkening gradually extended forward and increased in intensity. A gonadectomized female (*bird 123*) reached a maximum color of the legs after 3 injections, of the lores and buccal cavity after 8 injections. A castrate male (*bird 113*) did not attain the same leg color until after 5 injections. Younger adults required a greater number of injections to secure the same result (table 2) but chicks with gonads intact responded quickly (table 3). In every case, however, full response was obtained by a dosage of 21.25 mg. or less, spread over a 3-week period.

A slight sex difference in the reaction to the androgen was shown by the castrate

birds. The males developed the black pigment of the lores, bill and buccal cavity more quickly than did the females. Conversely, the latter retained the purple stage in the coloring of the lores for a longer period than did the males. Most of the females, however, developed red legs more quickly than did the males when both were receiving the same amount of testosterone propionate.

Although there was considerable individual variation in the treated birds, the difference between experimentals and controls at the close of the treatment period was always marked. The difference between two typical pairs of one and one-half-

TABLE 3. EFFECT OF TESTOSTERONE PROPIONATE ON THE SEXUAL PIGMENTATION OF BLACK-CROWNED NIGHT HERON CHICKS

Preparation	Sex and bird No.	Age	Duration of exp.	Dosage	Amt. injected	Remarks
		days		cc.	cc.	
Perandren	♀ 6	20	6/9–6/28	0.25	3.5=17,500 γ	After 2 inject., complete breeding pigmentation indicated. Coloration of feet lagging, probably because masked by intense green color of juvenile bird. After 3 inject., voice guttural.
	♂ 16	20	6/9–6/28	0.25	3.5=17,500 γ	After 2 inject., complete breeding pigmentation indicated. Coloration of feet lagging, probably because masked by intense green color of juvenile bird. After 3 inject., voice guttural.
	♀ 26	20	6/9–6/28	0.25	3.5=17,500 γ	After 3 inject., voice guttural. After 8 inject., pigmentation complete.
	♀ 36	20	6/9–6/28	0.25	3.5=17,500 γ	After 3 inject., voice guttural. After 7 inject., feet show fair amount of red masked by green. After 10 inject., pigmentation complete.

year-old herons may be shown by reference to the "color standards" of Maerz and Paul (5). The untreated gonadectomized adults had the following colors.

♀ *112*—legs, Pl. 29 C 5; lore, Pl. 35 E 6

♂ *121*—legs, Pl. 28 C 3; lore, Pl. 36 E 8

The perandren-treated pair at the close of the treatment period had the following.

♀ *123*—legs, Pl. 6 F 4; lore Pl. 47 E 10

♂ *113*—legs, Pl. 5 E 4; lore Pl. 47 A 9

In striking contrast, none of the estrogens was found to have any effect on the color change of head or legs (tables 1 and 2). Although the dosage was adequate to produce a marked hypertrophy of the Müllerian duct, it failed to produce any sexual color on lores, bill or legs. Even 50,000 I.U. of estrone acetate, administered over a period of 15 days during August to an adult female (*bird 406*) with ovary intact, failed to produce any evidence of sexual color, although the oviduct became considerably enlarged and vascularized (table 4).

The effect of testosterone propionate on the legs of both sexes of the night heron was to increase the vascularization of the integument. This could be readily shown by squeezing the legs of the treated bird. The blood would flow from the superficial blood vessels restoring the original pale greenish tinge to the integument. Many birds, when frightened or roughly handled, would lose the pink tones of their legs temporarily.

Sections of the integument from the legs of treated and control birds disclosed a marked difference in their vascularity (fig. 3, 4). The superficial blood vessels of the dermis of the treated bird were increased in size and at various points pushed slightly into the germinative layer of the epidermis. The epidermis of these injected birds was more active. Nuclei in the layer immediately distal to the germinative layer were not flattened, as in the control birds, but of nearly the same width as the nuclei of the

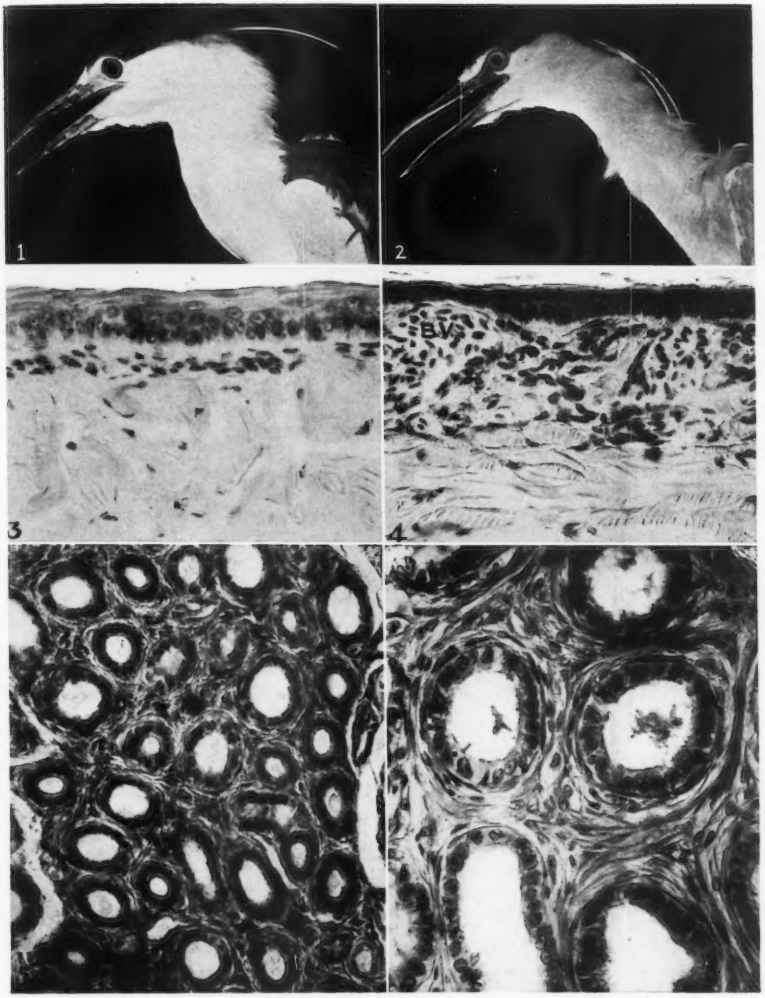

Fig. 1. CONTROL BLACK-CROWNED NIGHT HERON; THE LORES ARE GREENISH-YELLOW. Fig. 2. EXPERIMENTAL AFTER INJECTION WITH TESTOSTERONE PROPIONATE; THE LORES ARE BLACKISH-BLUE. Fig. 3. INTEGUMENT FROM LEG OF CONTROL BIRD. ×360. Fig. 4. INTEGUMENT FROM LEG OF BIRD INJECTED WITH TESTOSTERONE PROPIONATE; AN ENORMOUS INCREASE IN THE SIZE AND NUMBER OF THE CAPILLARIES AND BLOOD VESSELS UNDERLYING THE EPIDERMIS HAS OCCURRED. (B V = Blood Vessel). Fig. 5. SECTION OF EPIDIDYMIS OF CASTRATED MALE NIGHT HERON (*422*) FOLLOWING INJECTION OF AMNIOTIN. ×370. Fig. 6. SECTION OF EPIDIDYMIS OF CASTRATED MALE NIGHT HERON (*113*) FOLLOWING INJECTION OF TESTOSTERONE PROPIONATE. ×370.

germinative layer. Further, they were not all horizontal but frequently vertical or irregularly arranged with relation to the outer surface. The horny layer of some injected birds was thinner but in other birds it was the same thickness of the controls. No increase in the number of pigment cells was noted in the injected bird.

In striking contrast, sections of the lore of the injected female (*bird 123*) showed an enormous increase in pigment below the epidermis. Masses of melanin surrounded the more superficial blood vessels of the dermis. Only a few scattered pigment cells were present in the untreated bird. Some pigment was found near the deeper blood vessels of the dermis in both the injected bird and the control. Thus the testosterone

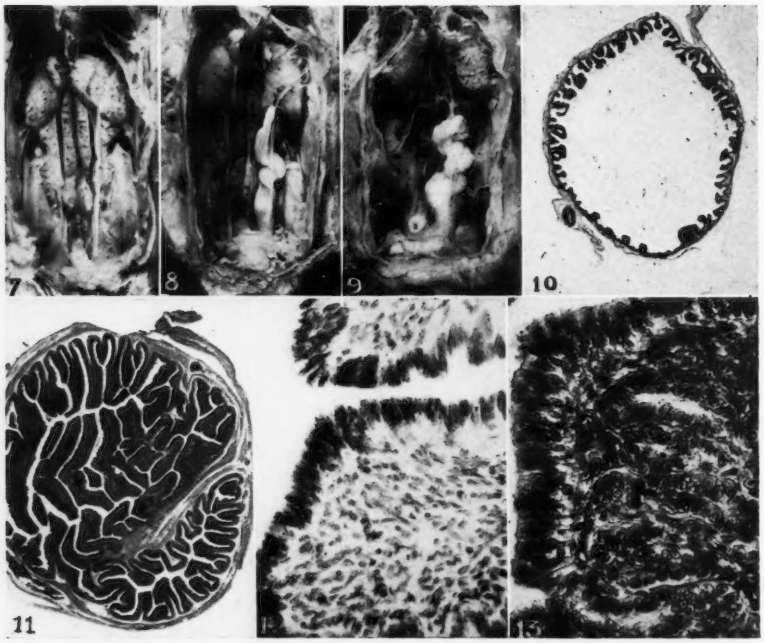

Fig. 7. Oviduct of spayed night heron (31) following injection of sheep whole pituitary gland. Fig. 8. Oviduct of spayed night heron (322) following injection of testosterone propionate. Fig. 9. Oviduct of spayed night heron (333) following injection of amniotin. Fig. 10. Cross section of oviduct of spayed night heron (322) following injection of testosterone propionate. ×24. Fig. 11. Cross section of oviduct of spayed night heron (333) following injection of amniotin. The hyperplasia of the mucosa is clearly shown. ×24. Fig. 12. A portion of the mucosa of the oviduct of a night heron (123) injected with testosterone propionate. ×314. Fig. 13. A portion of the mucosa of the oviduct of a night heron (333) injected with amniotin. The enormous development of the glands is well shown (compare fig. 12). ×314.

propionate increased the pigment, not uniformly throughout the dermis but chiefly in its superficial layers.

Effect of estrogens and androgens on the genital tract. Gonadectomy experiments gave further support to the view that the ovary normally secretes male hormone. In the adult female there is retained a rudimentary Wolffian duct. In spayed females, treated with estrogens, this remains small. The epoophoron forms an enlargement at the anterior end of this duct. In spayed females, including those treated with estrogens (table 3), this epoophoron varied from 0.6 to 1.5 mm. in greatest width. In control females, killed at the height of the breeding season, this was 2 mm., or slightly more, in width. This is the same width it attained in most spayed females when treated with testosterone propionate (table 4).

The homologous epididymis was larger both in the breeding male and in the castrate, treated with testosterone propionate. In 4 breeding males the epididymis at its thickest point was 4 mm. wide and 2.7 mm. at its narrowest point. The average width of the 8 epididymides was 3.1 mm. Further, the epididymis was formed of more tubules than was the epoophoron. Sections of the epididymis of the castrate, treated with testosterone propionate, and its control showed a marked difference in structure. The columnar epithelium was relatively high in the testosterone propionate

TABLE 4. EFFECT OF ESTROGENS AND ANDROGENS ON THE GENITAL TRACT OF THE BLACK-CROWNED NIGHT HERON

Preparation	Sex and bird No.	Shell gland	Av. width oviduct	Rt. epid. or epooph.	Lt. epid. or epooph.	Lt. gonad	Rt. gonad
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Pituitary	♂ *R2*	—	—	1.5	1.0	castrated	
	♀ *31*	3.0	1.5	1.0	1.0	spayed	
Normal	♀ *112*	4.0	4.0	0.9	0.6	spayed	
	♀ *Y-B*	2.5	2.8	—	—	spayed	
	♂ *121*	—	—	2.0	2.0	castrated	
	♂ *Y135*	—	—	0.7	1.0	7 ×3	6.5×3
Estrogens—							
Estrone acetate	♀ *406*	11.5	10.0	1.5	1.5	14.5×6	—
Theelin	♀ *Y-GG*	10.0	6.0	1.1	0.7	spayed	
Amniotin	♀ *333*	9.0	6.0	1.0	1.0	spayed	
Theelin	♂ *311*	—	—	0.8	1.1	castrated	
Amniotin	♂ *422*	—	—	1.0	1.0	castrated	
Androgen—							
	♀ *322*	5.0	4.0	2.0	2.5	spayed	
Perandren	♀ *123*	5.0	3.0	2.0	1.5	spayed	
	♀ *Y-B*	7.0	6.5	2.0	2.0	spayed	
	♀ *204*	6.5	5.0	2.0	1.2	20 ×6	—
	♀ *214*	6.0	5.0	1.5	1.5	21 ×7	—
	♀ *141*	4.0	2.5	1.5	1.2	18.5×6.5	—
	♀ *14*	5.5	4.0	1.9	1.1	12 ×7	
	♀ *Y131*	4.5	2.5	2.0	1.5	15 ×7	
	♂ *411*	—	—	3.0	3.0	castrated	
	♂ *113*	—	—	2.5	3.9	castrated	
	♂ *344*	—	—	2.5	2.5	9 ×3	9 ×3
	♂ *41*	—	—	2.5	2.5	8.5×3	10.7×3
	♂ *34*	—	—	2.5	2.5	9 ×4	10 ×4
	♂ *101*	—	—	3.0	4.0	11.5×4	11.5×4.5
	♂ *103*	—	—	3.9	3.9	10 ×5	10 ×5

treated bird (♂ *113*). Further, the cells of the epithelium had granular cytoplasm and relatively pale nuclei. The lumen of the tubules was relatively larger than in the control and contained some secretion (fig. 6). Sections of the epoophoron of the spayed female, treated with testosterone propionate, showed a similar condition of the epithelium. The epithelium of the epididymis of a control (♂ *121*) had less granular cytoplasm and dark or pycnotic nuclei.

The epididymides of male herons treated with the estrogens showed various degrees of degeneration and in none was the epithelium hypertrophied as in the testosterone propionate treated bird (fig. 5). Male herons, of the ages employed in this study, showed no evidence of Müllerian ducts. Females treated with estrogens showed no hypertrophy of the epoophoron, although the Müllerian duct was greatly enlarged.

It is interesting that the night heron differs conspicuously from the starling, as described by Witschi and Miller (3), in that the Müllerian ducts of both immature and adult females are enlarged by testosterone propionate (table 3). This enlargement (fig. 8), however, was due merely to a distention of the ducts without a marked hyperplasia of their mucosa (fig. 10, 12). In the female, injected with an estrogen, the lumen of the duct was nearly filled by the highly convoluted lining and glandular hypertrophy. This is well shown in sections of the oviduct of the amniotin treated bird (fig. 11, 13). In none of the spayed females, however, did the dosage of estrogen employed here hypertrophy the Müllerian duct to the extreme degree found in the breeding bird. Immediately after egg laying the Müllerian duct of one untreated female was 17 mm. wide at its broadest point, the shell gland, and averaged 9.1 mm. in width. The injections of estrone acetate enlarged the Müllerian duct of a noncastrate bird until it measured 11.5 mm. at its thickest part. As shown in table 4, other estrogens (fig. 9) did not hypertrophy the Müllerian ducts of other females to this degree.

On the other hand, the Wolffian duct and epoophoron of the treated female night heron enlarged to the same degree as that found in the breeding bird. For example, in the normal female (*bird 131*) taken at the height of the breeding season the epoophoron was 2 mm. wide. This represents practically the same degree of hypertrophy attained in our spayed females treated with testosterone propionate (table 4). Since estrogens do not have this effect it follows that the normal seasonal hypertrophy of the Wolffian duct and epoophoron in the night heron is produced by an androgen secreted by the female during the breeding season.

Effect of testosterone propionate on behavior. The courtship behavior of the black-crowned night heron has been fully described by Noble, Wurm and Schmidt (1). Both sexes develop at the beginning of the breeding season a guttural voice. Both sexes overture, that is, snap the bill while lowering the head and giving a recognition call. During this gesture the plumes are erected and the breast and, usually, the neck feathers are erected. Both sexes narrow the pupils and protrude the eyeballs while displaying in this fashion. The male goes beyond the female in erecting the breast feathers to a greater extent. Although both sexes play with sticks, the male is distinctive in developing a "stick ceremony." During this performance the head is raised very high and the mandibles snapped on a stick which is held between them and close to the body.

The most distinctive feature of the male's courtship is his "snap-hiss." During this ceremony the head is extended forward and downward, followed by snap and hiss sounds, given while the legs are alternately lifted and replaced. This ceremony serves to attract females to the territory held by a courting male. Until the present season it was thought that an untreated female under no circumstances develops this snap-hiss ceremony, but twice on June 12 and twice on June 14, 1939, a single female (*bird 1224*) displayed this ceremony. The bird later submitted to copulation and laid eggs. On August 11, its ovary was found to be normal. This exceptional bird was presumably producing an abnormal amount of androgen. At least, no other untreated female in our colony of breeding birds ever showed this behavior.

As shown in table 5, injections of testosterone propionate induced the complete male behavior in the adult or immature female. The first effect of the injections following the increased pigmentation was the development of a guttural voice. This appeared when the bird had received from 7 to 10 mg. of testosterone propionate. It is most readily demonstrated by picking up the bird. Then the protest call, a raucous

screech, will be found to have given way to a gasping, guttural note, given with wide open bill. Gonadectomized females required more testosterone propionate to produce this change of voice than did gonadectomized males, but immature birds showed little sex difference (table 3). Soon after the change of voice, the exophthalmic condition of the eyes during courtship activity becomes evident. If treated birds are released in this stage they will show a particular interest in sticks and will seek and defend old nests when these include suitable nesting material and nest sites. Then both sexes of treated birds follow with male twig ceremony, male breast feather display, and typical snap-hissing. No one bird was observed to give every detail of

TABLE 5. EFFECT OF TESTOSTERONE PROPIONATE ON THE BEHAVIOR OF THE BLACK-CROWNED NIGHT HERON

Amt. injected	Age	Sex	Bird No.	Modifications of behavior
27,500 γ	1½ yrs.	♀	141	Male twig playing; snap-hiss.
27,500 γ	Immat.	♀	204	Male twig playing; snap-hiss walk (no sound); snap-hiss; male breast feather fanning.
27,500 γ	Immat.	♀	214	Male twig playing; snap-hiss; male breast feather fanning; extensive territory defense; no reaction to ♂ 21 courtship.
15,000 γ	Immat.	♀	213	Male twig playing; snap-hiss; male breast feather fanning; extensive territory defense; nest building; copulation ♀ 131.
20,000 γ	Adult	♀-Gx	31	Male twig playing; snap-hiss; male breast feather fanning.
15,000 γ	Adult	♀	11	Male twig playing; snap-hiss.
20,000 γ	Adult	♀	33	Male twig playing; snap-hiss; extensive territory defense; attacked by original mate ♂ 21—failure to act as ♀.
22,500 γ	20-day chick	♀	6	Male twig playing; male breast feather fanning; extensive territory defense; male courtship repulsed by ♂ 16.
22,500 γ	20-day chick	♀	26	Male twig playing (incomplete); nest building; incubation; submitted to copulation.
22,500 γ	20-day chick	♀	36	Male twig playing (also incomplete); male breast feather fanning; extensive territory defense; male courtship repulsed by ♂ 16; attacks ♂ 16 while courting; copulates ♂ 16; twig carrying; nest building; simulates feeding of young.
27,500 γ	Adult	♂	41	Male twig playing; snap-hiss; male breast feather fanning; extensive territory defense.
27,500 γ	Adult	♂	34	Male twig playing; snap-hiss.
21,250 γ	Immat.	♂-Gx	411	Male twig playing; snap-hiss; extensive territory defense.
21,250 γ	20-day chick	♂	16	Male twig playing; male breast feather fanning; extensive territory defense; copulation ♀ 36; ♀ 26; submitted to copulation by ♀ 36; nest building and twig carrying; incubation.

Gx = gonadectomized.

male courtship behavior, as previously described, probably because it was not possible to have the birds under continuous observation. Nevertheless, as shown in table 5, some distinctive male behavior was exhibited by all of the injected females and the group as a whole gives a complete picture of male courtship. One stimulated female, 213, was observed to mount and attempt to copulate with another female.

Although the testosterone propionate induced female herons of a wide variety of ages to give the snap-hiss ceremony, copulation attempts did not follow at once. As shown by Noble, Wurm and Schmidt (1), copulation normally follows only after a period of adjustment during which the male secures sexual dominance over the female. Thus among the treated adults, the males, paired with females 11 and 33, attempted to mount but the females would not submit and copulation failed. It is

possible that the injections had carried them too far in the male direction to permit a ready adjustment.

Further evidence that the sexual dominance relations require time for adjustment was shown by the behavior of the 3 female chicks, *6*, *36* and *26*, and one male chick, *16*, which were only 20 days old at the beginning of the experiment. These 4 birds were not gonadectomized. When given the same amounts of testosterone propionate the male responded first. When the 4 were released in a large flying cage with other night herons, they formed a single group and did not pair off at once. Although the female, *bird 6*, dominated the group at first, ♂*16* within a few days drove ♀ 6 out of the roosting territory. On the floor, however, away from the nesting material, there was little antagonism between ♀ 6 and ♂*16*. The least reactive bird of this series, ♀ *26*, never developed complete male twig ceremony, although it played with sticks while nest building. While ♀ 6, which had developed male twig ceremony and male breast feather display, was driven out of the group, the more submissive female, *bird 26*, was accepted and assumed the subordinate rôle in copulation with ♂*16*.

The further history of this group gave additional evidence of the importance of the time factor in securing adjustment. After ♀ 6 had been driven out of the group, ♂*16* copulated with ♀ *36* several times. Later, pecking was observed between these two birds, giving evidence of a maladjustment. On the same day ♀ *36* mounted ♂*16*, and performed a typical copulation. The male overtured to the female following this copulation and this may be considered further proof that he had accepted a subordinate rôle. A subsequent pecking bout during a courtship display indicated that the dominance was not complete. This was soon followed by ♂*16* regaining the higher head position and copulating with the female on succeeding days. Testosterone propionate may, therefore, raise a female to a sexually dominant position permitting her to assume the dominant rôle in copulation. If the hormone level is not sustained the female may drop to the subordinate position and this shift is indicated by a period of maladjustment during which there is fighting, combined with billing, as each bird endeavors to keep a superior head position.

Since the treatment forced sexual activity so quickly on this group of young birds, there was never a typical pairing off. Male *16* continued to pay attention to ♀ *26* and joined her in incubating an egg left in a nest by a pair of adult breeding herons, which the young birds succeeded in evicting. Hence, the testosterone propionate had given this pair of chicks such a great interest in nesting material that they could drive off a pair of adults which out of the breeding season would normally dominate them.

These observations on gonadectomized adult birds, on immature and very young herons indicate that the androgen, testosterone propionate, will induce the behavior common to both sexes, namely, a search for territory which includes nesting material, also a mutual courtship including a feather display. If the androgen is given in sufficient amounts, the distinctive male behavior will also appear in both sexes. This includes the twig and snap-hiss ceremonies. It also embraces a sexual dominance, which is necessary before copulation can proceed. A subordinate male, although potentially able to copulate, must gain a dominant position during a billing bout before he can mount his mate.

Since no estrogen was found to have any effect on behavior, it would seem that the sexual behavior of the night heron is controlled entirely by androgens. The estrogen produced by the ovary has important functions in preparing the oviduct for the passage of the eggs. It would not seem to have any rôle in regulating the social adjustment which eventually leads to a fertilization of the eggs.

DISCUSSION

Testosterone propionate has been found to produce crowing and aggressiveness in chicks (6), crowing in hens (7) and singing in female canaries (8). Shoemaker (9) induced not only singing in his female canaries with testosterone propionate but also 3 features of male courtship: *a*) singing close to the head of the control birds, *b*) posturing with neck outstretched and body thin and *c*) dangling nesting material before other females. This courtship did not proceed to copulation which is the more surprising in that two of Shoemaker's untreated females copulated like males. Prepuberally castrated rats and guinea pigs injected with testosterone propionate will copulate freely (10, 11). Further, female mice which had been made intersexual by injecting their mother before parturition with a male hormone developed a typical male mating behavior when treated with testosterone propionate (12). In our experiments the testosterone propionate not only carried the night heron chicks through copulation but even induced brooding. This final phase probably involved other hormones and presumably prolactin from the pituitary.

We have found that certain male heron chicks developed a black lore quicker than the females did. This may mean not a difference in sensitivity of the sexes to the androgen but merely that the immature testis is also producing some androgen. Frank, Klempner and Hollander (13) demonstrated that comb growth was greater in male than in female chicks treated with androsterone, but Breneman (14) found that the testis, even in the 10 to 15-day old chick, is probably releasing some androgen because the partially caponized birds had larger combs than had the complete capons.

The cyclic color change in the legs of the night heron is not unique among birds. Levi (15) presented evidence that the red color of the legs of the breeding male *Vidua chalybeata* was induced by a testicular hormone but he does not make clear if this is a true pigmentation or a mere vascularization as in the night heron. It would seem that the androgen in the latter species acted on the autonomic system for not only are the capillaries expanded but the eyes bulge and the pupils contract. But in castrated rats, testosterone increases the size of livers, kidneys and hearts (16) and in the female guinea pig it produces a considerable increase in the temporal muscles (17). It is probable that the androgen secreted by the night heron during the breeding season has a multiplicity of effects on the body.

The source of the androgen secreted by the ovary of the night heron is presumably in the ovary since our gonadectomized birds developed no secondary sexual characters. The experiments of Steinach and Kun (18) and Guyenot, Ponse and Wietrzykowska (19) show that the ovary of the guinea pig can, under certain conditions, have a distinct androgenous effect. Lamar (20) and Greene, Burrill and Ivy (21) have shown that progesterone in large amounts is androgenic in the rat. Mice ovaries, grafted to the ears of castrate males, can restore the atrophy of the seminal vesicles resulting from castration (22). In the rat, however, intense luteinization of the ovary has no influence on the structure of the prostate and seminal vesicles. This led Desclin (23) to doubt that there is any relation between luteinization and the production of an androgen in this form. Domm (24) reported a masculinization of the newly hatched chick by treatment with anterior pituitary extract. This was correlated with a marked hypertrophy of the medullary portion of the ovary. Greenwood and Blyth (25) suggest that the sex cords arising from the germinal epithelium of the fowl's gonad produce androgens and the secondary cords, estrogens. Male hormone has been detected, however, in the urine of castrated animals, indicating an extragonadal source (26). The adrenals may play a rôle in the elaboration of this androgen, as has been shown in castrate rats (27).

The cyclic appearance of the secondary sexual characters of the night heron has been shown above to be due to an androgen in both sexes. In the domestic fowl, the hen's comb owes its size primarily to the amount of androgen present in the tissues. In birds, such as the starling (3) and the night heron, where the epigenetic characters are nearly equally developed in both sexes, it does not follow that the androgen level is the same in both sexes. As shown above, the darkening of the lore appears early and indicates only that a certain minimum amount of androgen is present. In the English sparrow the dark pigmentation of the male's bill during the breeding season is regulated by an androgen (28). The female sparrow reacts slower to the male hormone and the pigmentation of the bill is never complete (29). In the night heron there is much less difference in the response of the two sexes.

Testosterone propionate enlarges both Wolffian and Müllerian ducts of the night heron. It also enlarges the Müllerian duct of the alligator (30) and of the pouch young of the opossum (31). The effect of testosterone propionate on the Müllerian duct of the alligator is to produce a mucosal hyperplasia of the same nature but not as extensive as that produced by an estrogen (30). This is very different from the night heron in which testosterone propionate enlarges the oviducts without producing any glandular hyperplasia.

There is abundant evidence that the effect of testosterone propionate is different from that of any estrogen in other animals. Burns (32) found no estrogenic effect of testosterone propionate in larval salamanders. It induces a cylindrical elongation and turning of the cloacal rim of the female canary which does not occur in the untreated female (9) even at the height of the breeding season. It leads to a growth of the penis in the Barbary duck, *Cairina*, and this is characteristic only of the male (33). Gardner and Pfeiffer (34) showed that testosterone propionate inhibits the pubic resorption in mice produced by estrogens. In spayed immature rats it can cause, however, an opening of the vagina (35). The response of the guinea pig endometrium to testosterone is qualitatively similar to the response to theelin although these two substances differ in potency with respect to this effect (36). The administration of testosterone to female rabbits leads to an enlargement of the uterus and progestational proliferation but Brooksby (37) performed traumatization experiments on the rat and found that the action of testosterone was qualitatively different from that of estrogens. Testosterone propionate suppresses the behavioral responses characteristic of heat and ovulation in the guinea pig and also prevents the opening of the vagina in this form (38). It also counteracts the muscle stimulating action of estrogens resulting in a suppression of the normal tubal contractions (39). In brief, the effect of testosterone is readily distinguished from that of estrogens in many animals.

The different responses of the genital ducts to testosterone propionate in the different groups of animals may be due to genetic differences. It is also possible that the chemical nature of the mother hormone is modified when circulating in the different animals (40). Willier, Rawles and Koch (41) have shown that the masculinizing effect of testosterone propionate is different from that of androsterone. Since variations in temperature have a great influence on the effect of androsterone on the capon's comb (42), it is to be expected that this androgen would have a different effect in warm and cold-blooded animals.

One of the most interesting conclusions of our study is that no phase of the female behavior is due to estrogens. Witschi (43) assumes that male behavior seen in a few female birds during the mating season is due to an androgen. Unfortunately the hormonal basis of breeding behavior is not known in any of these species. Further,

the only other bird besides the heron which has the breeding colors nearly equally developed in both sexes and under the control of an androgen is the starling. In this species, as shown by Freitag (44), the courtship behavior of the male is readily distinguished from that of the female. Whether such features as the collecting of brightly colored leaves and flowers to place in the nest platform is due to a high level of androgen in the male sex is not known but in view of the work on the night heron it seems probable.

The testosterone propionate aroused a mutual interest of the night herons in nesting material but the birds which received more of the androgen went further in the courtship with the result that pairs formed. In other birds it is possible that estrogens have a more definite rôle in sexual behavior. Dantchakoff (45) found that a chick, genetically a male, when treated with an estrogen developed a rivalry to females and would accept food from a rooster. Nevertheless, copulation rarely occurred. In mammals, both estrogen and progesterone are involved in the full estrous response, at least in guinea pigs (46) and rats (47). However, progynon benzoate has been found to produce typical female behavior in male rats, both castrated and non-castrated (48), when these were not treated until they had lost their sex drive.

Ball (49) found that the sex activity of castrate rats was increased above the castrate level by an estrogen when these rats were treated before they had completely lost their sex drive. This suggests that the function of the male hormone in the adult is not to organize but merely to activate a pattern already laid down either genetically or under the previous influence of the gonads or androgenic substances in the adrenals. Our experiments with the effect of estrogens on the night heron showed that these had no influence on activating sex behavior. Rather, the difference between male and female behavior was regulated by the amount of androgen in the tissues of the two sexes. The fact that at the beginning of courtship both sexes are reacting to small amounts of the same hormone makes it not surprising that they have the same interest in nesting material and hence in a breeding territory. Only later do increasing amounts of the androgen shift the male's behavior pattern in such a way that pairs can form and copulation follow.

SUMMARY

Both sexes of the black-crowned night heron undergo a marked increase in the pigmentation of lore, lower mandible and buccal cavity during the breeding season. The legs also undergo an intense vascularization which gives them a pink color. Gonadectomy causes a loss of these modifications. Injections of testosterone propionate (Ciba) will produce these sexual changes in immature herons and in gonadectomized adults. Estrogens (theelin, estrone acetate, amniotin and follutein) do not have this effect. It is concluded that the secondary sexual characters of the night heron are presumably produced by an androgen in both sexes.

Both testosterone propionate and the estrogens cause an enlargement of the oviduct of the spayed night heron but only the estrogens produce a hyperplasia of its mucosa. The epididymides and their homologues are hypertrophied by testosterone propionate but not by the estrogens.

Testosterone propionate will induce male sexual behavior in adult females or in immature birds of both sexes. Even in month-old chicks it will make the voice more guttural and will induce territory defense, nest building, all male courtship ceremonies, copulation and, later, brooding. Differences between the sexual behavior of the adults seem regulated only by differences in the amounts of male hormone

normally found in these birds. Estrogens alone fail to stimulate any breeding behavior in either sex.

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THE SYNERGISTIC ACTION OF THYROID ON FLUORINE TOXICITY[1]

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IT HAS BEEN SHOWN by DeEds and Thomas (1) and by other investigators (2, 3) that a concentration of 0.0014% fluorine, corresponding to 14 parts per million, added to normal diet will produce a distinctly noticeable degree of bleaching of the incisor teeth in 100% of the rats studied. Largely because of the interest in the relationship of mottled enamel to the fluorine content of drinking water, and the potentialities for producing the same tooth defect by spray residues containing fluorine, it has become customary to refer to toxic amounts of fluorine in terms of parts per million. Fluorine spray residue tolerance limits are expressed in this manner. However, it seems apparent that the absolute amount of fluorine ingested might be of more importance than the concentration of fluorine present in one or more constituents of the diet. If rats could be made to eat more food than the normal daily intake, then a diet which contained slightly too little fluorine to cause bleaching of the incisors under ordinary conditions might result in the production of bleached teeth. The concentration of fluorine in the diet would remain the same, but the absolute amount of fluorine ingested would be increased.

The experiments described in this paper were designed to secure data on this question, and at the same time secure information which might be helpful in understanding the mechanism by which cadmium, when added to a normal rat diet as the chloride, produces a marked bleaching of rat incisors. The bleaching of rat incisors by cadmium is a phenomenon observed in this laboratory and will be reported elsewhere in studies on cadmium toxicity.

It is known that the feeding of thyroid will cause an increase in food intake, and it has been shown by MacKay and Callaway (4) that the daily injection of protamine zinc insulin will lead to a marked overeating by rats, resulting in very obese animals. Both of these procedures were used by us.

The animals used in these experiments were albino male rats, those in the first series weighing 65 to 70 gm. Three groups of 5 rats each were employed. The first group served as controls, and the second group received the same diet in which dried thyroid was mixed in a concentration of 0.3% for 25 days and in a concentration of 0.1% for the remainder of the experimental period. The third group received daily or twice-daily injections of protamine Zn insulin. The basic diet was the usual one employed in this laboratory, and contained corn meal, linseed oil-cake meal, alfalfa, casein, sardine oil, bone ash and sodium chloride. Since no sodium fluoride was added, the fluorine present must have had its source in the various dietary constituents, especially the bone ash. Fluorine determinations made on this diet on several occasions have shown a native fluorine content varying from 10 to 17 parts per million.

[1] Food Research Division Contribution No. 456.

A brief description of the color changes of the incisors of a normal rat from weaning to adult age will aid in an understanding of the results obtained in these and subsequent experiments. When the incisors of the young rat first erupt they are completely white. The anterior enamel surface of the incisors soon acquires a light brownish tinge, which rapidly darkens until it nearly reaches the depth of color seen in the incisors of the normal adult rat. The final increase in coloration is a slow process. Figure 1 shows the color change seen on the incisors of normal rats.

In figure 1 +++ represents complete bleaching of the incisor teeth, − indicates the brown color seen on the tooth of the normal adult albino rat, while ++, +, and ± correspond to intermediate degrees of bleaching. This arbitrary evalua-

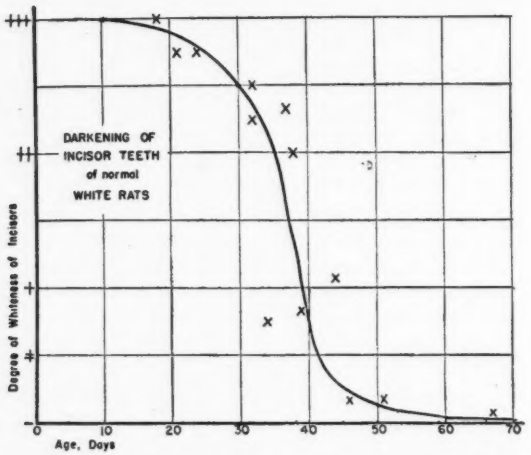

Fig. 1. Rate of darkening of incisor teeth of normal albino rats.

tion of the degree of bleaching permitted the observer to closely duplicate his estimation of the amount of bleaching even when not knowing which animal was being examined, and the estimation of one observer would agree well with that of another. For purposes of plotting, the plusses assigned to the individual animals in each group were averaged.

All the rats in the group receiving thyroid showed an early and lasting difference from those on the control diet. The rate of increase in body weight was considerably less than normal in spite of their greater food intake. Within 10 days, the incisors of the rats receiving thyroid were less colored than those of the controls, and after 20 days on the diet, were nearly white, while the incisors of the control animals had become as brown as is usual for the normal adult rat. This difference between control rats and those receiving thyroid was maintained till the end of the experiment, 75 days after the start.

A discussion of the probable mechanism involved in this difference between the 2 groups will be taken up after describing other experiments. The points to be emphasized here are that no fluorine had been added to the basic diet, that any fluorine present was native to the dietary constituents, and that normal rats placed on this diet without added thyroid did not retain bleached teeth into adult life. The inference to be drawn from this experiment is that, although the fluorine native to the diet does not differ in physiological action from that of added fluorine in the form of sodium fluoride, its concentration is not great enough to elicit the typical evidence of

a toxic action in the normal rat. However, under the influence of an induced pathological state of hyperthyroidism, the native fluorine produces the typical effect on the rat incisors. This point cannot be emphasized too strongly in view of arguments advanced to show that experiments based on the toxic action of fluorine added to a diet should not be used as evidence of the potential toxicity of native fluorine in the diet.

As pointed out in the introduction, 0.0014% of fluorine added to the basic diet has been shown to produce a noticeable degree of bleaching in 100% of the rats studied. In an experiment to be reported in detail elsewhere 0.0014% fluorine was added to the usual basic diet from which the bone ash had been omitted because it was the principal source of native fluorine. In lieu of the bone ash the calcium, magnesium and phosphorus were supplied in the form of c.p. inorganic salts. The feeding of this diet to albino rats did not result in the production of bleached teeth in more than a few of the rats. In other words the effective concentration of physiologically active fluorine must be the sum of 0.0014% added fluorine plus the native fluorine principally in the form of bone ash. If further support for the argument is needed it will be found in the following experiments which better represent conditions for a study of the main problem of this paper.

In the experiments described above no conclusions could be drawn from the data on the rats receiving protamine Zn insulin. In spite of great care with the animals, and a very slow increase in the insulin dosage, all of the animals died in convulsions within a few weeks of the start of the experiment. Believing that the carbohydrate of the diet was not sufficiently available to prevent the development of fatal hypoglycemia, a new dietary mixture was tried, using 90% of the above basic diet and 10% glucose. The animals on this diet survived no better than those in the first series. The suggestion was made by Dr. E. M. MacKay that the diet employed was too bulky to permit the consumption of a carbohydrate intake adequate to offset hypoglycemia, and that the diet employed in his experiments would be more suitable. The following experiments were therefore carried out.

The MacKay diet (4) contains 25% casein, 40% cornstarch, 15% butter fat, 10% lard, 5% brewer's yeast, and 5% salt mixture. In preparing this diet we used the Osborne-Mendel salt mixture, omitting the sodium fluoride, and since the other constituents contribute only minimal quantities of fluorine, the resulting basic diet presumably contained little fluorine. To various batches of this basic diet sodium fluoride was added to provide diets containing added fluorine in final concentrations of 0, 5, 10, 20 and 40 parts per million. These diets were fed to albino rats and the teeth were observed, the object being a determination of the concentration of added fluorine required to produce a noticeable degree of bleaching of the incisors in some, but not all, of the animals. The diet containing 10 parts per million, or 0.001% of added fluorine, was found to have the desired concentration, and was used as the basic diet for the remainder of the experiments.

Three groups of albino male rats averaging 50 gm. per rat were used. The first group served as controls, the second group received the same diet, but with the addition of 0.2% dried thyroid, and the third group received twice daily injections of protamine Zn insulin. Both thyroid and protamine Zn insulin caused a greatly increased food intake, especially so in the case of insulin. The effects on food intake and body weights are shown in figure 2.

Due to the greater metabolism, the rats receiving thyroid gained weight more slowly than the controls in spite of their greater food consumption. Autopsy of the rats receiving insulin showed that the animals had deposited excessive amounts of fat. The degrees of bleaching of the rat incisors of all groups are shown in figure 3.

The rats receiving injections of protamine Zn insulin ate about one and one-half times as much food as the controls and therefore ingested one and one-half times as much fluorine. However, this increment of 5 parts per million in fluorine intake did not cause any appreciable increase of bleaching of the incisors. Obviously it would require a large series of animals to distinguish between 2 diets in which the fluorine content did not differ by more than 0.0005%. The results obtained in the rats receiving thyroid contrasted sharply with the protamine Zn insulin results. Although the food intake, and hence the fluorine intake, was increased by thyroid, the increase

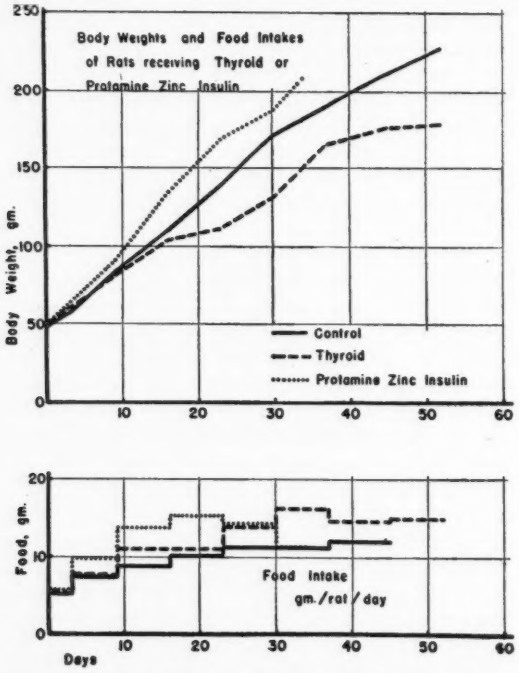

Fig. 2. EFFECT OF THYROID AND PROTAMINE ZINC INSULIN ON GROWTH AND FOOD INTAKE.

was less than that produced by protamine Zn insulin. However, the color of the teeth started deviating sharply from the normal in about 10 days, and within a month were almost completely white. The results obtained agreed well with those in the first experiment. The results obtained by the 2 methods employed to increase the food intake strongly suggest that the marked bleaching of incisors in rats receiving thyroid is not due to the increased fluorine intake. Moreover, as the result of experience extending over several years, it is known that it requires several times as much fluorine to completely bleach rat incisors as is needed to produce slight bleaching. The fluorine intake of the thyroid-fed rats was less than double that of the controls. Apparently the enhanced bleaching noted in the thyroid-fed rats is related to a specific effect of the thyroid independent of the effect on food intake.

At least two explanations may be advanced to account for the effect of thyroid feeding on fluorine toxicity. One explanation might involve a disturbance in balance between thyroid and parathyroid activity. Hammett (5) has reported that rats

which have been thyro-parathyroidectomized developed normal teeth, whereas the teeth were defective in rats in which only the parathyroid was removed. A more attractive explanation is related to the growth stimulation produced by thyroid. In normal rats and rabbits the lower incisors grow more rapidly than do the upper, the latter growing about two-thirds the rate of the former. It is a repeatedly confirmed generalization that young or rapidly growing animals or tissues respond more readily to the toxic action of a variety of agents than do mature animals. In rats fluorine bleaching occurs earlier or on a lower dosage on the rapidly growing lower incisors

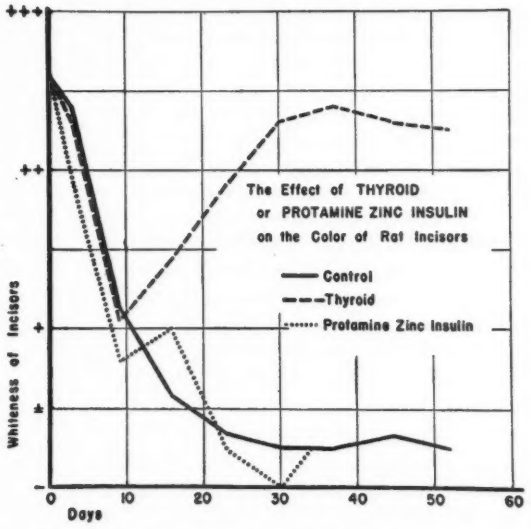

Fig. 3. Effect of continued administration of thyroid and protamine zinc insulin on the color of rat incisors.

than on the slower growing upper incisors. Karnofsky and Cronkite (6) recently reported that the rate of incisor tooth eruption in rats is markedly accelerated by the injection of thyroxin. Their observations in conjunction with those of the authors may mean that the toxic action of a given dosage of fluorine is enhanced by the increased rate of differentiation and growth of the organism produced by feeding thyroid. In other words an induced pathological state of hyperthyroidism has rendered the organism more susceptible to a given level of fluorine intake.

This pronounced synergistic action of thyroid on fluorine poisoning is not the first claim of a relationship between the thyroid gland and the physiological action of fluorine. As early as 1854 Maumené (7) claimed to have produced a struma-like swelling in the throat of a dog by the administration of about 10 gm. of sodium fluoride over a period of 4 months, and suggested that a high content of fluorine in drinking water might cause endemic struma. Independently Goldemberg (8) made the same suggestion in 1921, and reported that the feeding to rats of 2 to 3 mg. of sodium fluoride daily for 6 to 8 months produced a 5 to 6-fold increase in the size of the thyroid gland. Similar results have been reported by Pighini (9). On the other hand, Tolle and Maynard (10) found no difference in weight between the thyroid glands of fluorine poisoned rats and control rats, and Chaneles (11), reporting observations on rats, found neither macroscopic nor microscopic changes of the thyroid gland. Chang and

coworkers (12) have reported that the long-continued feeding of fluorine to cows nearly doubles the normal low fluorine content of the organs, but that the fluorine content of the thyroid may increase 240-fold.[2] The nearest approach to the synergism reported herein is the report of Phillips and coworkers (13) that the feeding of desiccated thyroid augments fluorine toxicity in the chick. Phillips (14) has also shown that the basal metabolism of rats is much greater, and that death is more frequent if both fluorine and thyroid are given to rats rather than thyroid alone, despite the fact that fluorine alone did not affect the basal metabolism. On the other hand, in studies on the fluorine content of the thyroid gland in cases of hyperthyroidism, Evans and Phillips (15) found no correlation between the fluorine content and the B.M.R., nor between the fluorine and iodine contents of the gland. Phillips and Lamb (16) have reported a mild parenchymatous proliferation in the thyroids of rats fed sodium fluoride, but observed no gross hypertrophy.

While the evidence of a relationship between fluorine and the production of goiter is contradictory, the potentiation of the fluorine bleaching of rat incisors by administration of thyroid is strikingly clearcut. This synergism is a striking instance of increased susceptibility to chronic poisoning brought about by an induced pathological state, and gives rise to several interesting questions. In areas where mottled enamel is endemic, does the severity of the tooth defect correlate with the B.M.R.? In regions where only a few indigenous individuals have mottled enamel, are those few the individuals who were hyperthyroid during the period of enamel formation? Should a spray residue tolerance limit for fluorine be set to protect the normal, the hyperthyroid, or the hypothyroid individual? Lastly should the tolerance limit take into consideration that in certain areas the public is already exposed to a fluorine intake in the drinking water?

SUMMARY

Control rats on the basic diets employed developed normal or nearly normal incisor teeth. Rats on the same basic diets to which dried thyroid had been added developed incisor teeth that were markedly bleached due to a potentiation of the toxic action of the fluorine in the basic diet. Subcutaneous injections in rats of protamine zinc insulin greatly increased the food consumption as reported by MacKay and Callaway. An increment in fluorine intake equivalent to 0.0005% fluorine in the diet, produced by increased food intake, did not increase the bleaching of rat incisors.

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[2] In the discussion by Chang, Phillips, Hart and Bohstedt (12), it is said that the fluorine content of the thyroid increased 24 times. A recent personal communication from Dr. Phillips stated that the values given in the table were correct, but the figure in the text should be 240.

CYTOLOGICAL EVIDENCE OF SECRETORY ACTIVITY IN THE MAMMALIAN PARATHYROID GLAND

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ALTHOUGH there is abundant clinical and experimental evidence of hormone production by the parathyroid gland, clear cytological evidence of secretory activity has not been forthcoming. Study of the human gland has demonstrated the presence of fatty inclusions, glycogen and occasionally pigment in the 'principal' cells, to which the essential rôle in hormone production is generally ascribed; in addition to these inclusions, no recognizable secretion antecedents have been observed. The 'oxyphile' cells are usually regarded as a degenerative form of the 'principal' cells; they are typically filled with acidophilic granules, which are commonly considered to be of no importance in the secretion of the gland. A large number of mammals has been investigated; the parathyroid usually consists of a single basic cell type, homologous with the 'principal' cell of man; 'oxyphile' cells are absent in the common laboratory mammals, but have been described in the cow and steer (1). The search for secretion antecedents in the mammalian parathyroid has been almost entirely unsuccessful. However, there are certain exceptions; and one should consult, among others, the papers of Schaper (2) (granules staining with iron hematoxylin, sheep), Rosof (3) (secretion antecedents in the form of osmiophile substance, albino rat), and Cowdry and Scott (4) (basophilic masses, monkey). It is fair to state that most workers consider that true secretion antecedents have not yet been demonstrated (5, 6).

The object of this report is to describe briefly some observations which appear to offer an important clue to the normal secretory mechanism of the mammalian parathyroid gland. These observations have been made upon the parathyroid glands of the Virginia and Barasingha deer (*Odocoileus virginianus borealis* and *Rucervus duvaucelli*). In both species the gland is composed of a single basic cell type, homologous with the 'principal' cell of man (7, 8).

Virginia deer. Specimen No. 1 was from a male, about 1½ years old. It was fixed in 10% neutral formalin. In examining ordinary hematoxylin and eosin preparations of the gland, one is immediately struck by the presence of numerous small basophilic granules in the cytoplasm of the parenchymal cells. Figures 1 and 2 give a far better idea of these granules than could be conveyed by a lengthy description. The field illustrated represents one of the heavier accumulations of the granules, but such accumulations are not at all uncommon. Some of the cells are entirely devoid of the granules, others contain them in only small numbers. The granules stain black with iron hematoxylin, and give a negative Millon's reaction. Specimen No. 2, from an adult male, was fixed in Zenker-formol. Similar granules were present in considerable numbers. With this fixative they did not stain well with ordinary hematoxylin, but were well stained with iron hematoxylin. The granules were on the whole less nuerous than in specimen 1, but in individual cells or groups of cells their number may be quite large. Specimen No. 3, from a female, about 1½ years old, was fixed in 10%

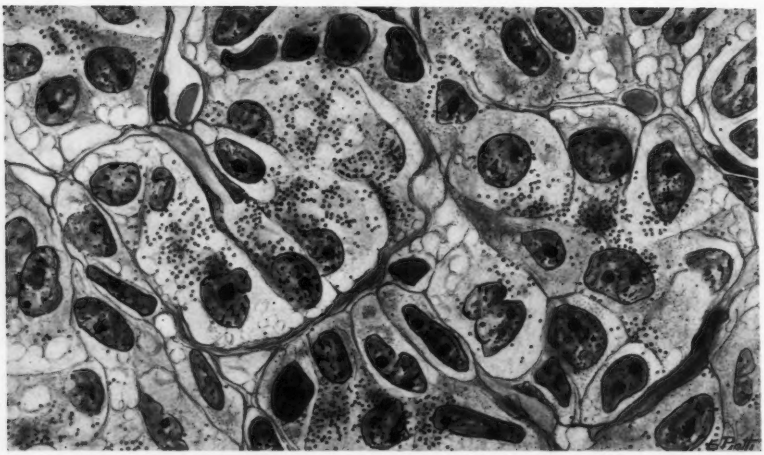

Fig. 1. PARATHYROID GLAND OF YOUNG MALE VIRGINIA DEER (specimen 1), ILLUSTRATING THE SIZE AND DISTRIBUTION OF BASOPHILIC GRANULES IN THE CYTOPLASM OF THE PARENCHYMAL CELLS. Paraffin section, 5μ, hematoxylin and eosin. Camera lucida drawing, $\times$1440.

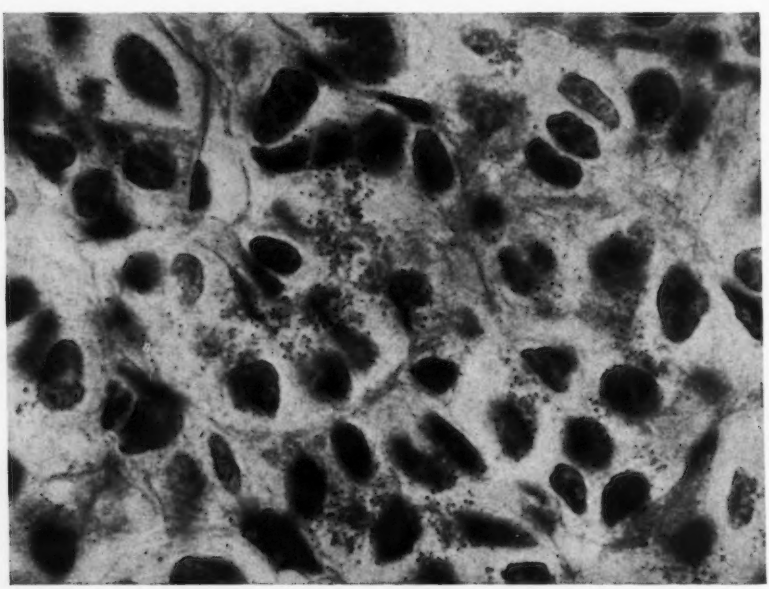

Fig. 2. PHOTOGRAPH INCLUDING THE SAME FIELD ILLUSTRATED IN FIGURE 1. $\times$1440.

neutral formalin and Zenker-formol. In this specimen the granules were likewise present in considerable numbers (comparable with specimen 2, less than in specimen 1). They showed up best in ordinary hematoxylin preparations of the formol material and in iron hematoxylin preparations of the Zenker material.

Barasingha deer. A specimen from an aged female was fixed in 10% neutral formalin. Small basophilic granules, entirely analogous to those observed in the Virginia deer, were present in the gland of this species in considerable numbers (comparable with specimens 2 and 3 above). The heaviest accumulations of these granules were equivalent to those illustrated in figures 1 and 2.

The pictures observed in the parathyroid gland of the deer are indeed striking when compared with the usual appearance of the 'principal' cells in man and other mammals, and certainly suggest a granular secretory mechanism for the elaboration of the hormone. It is to be emphasized, however, that there is no proof that the basophilic granules here described actually represent the secretion antecedent of the hormone. The opportunity has presented itself to obtain specimens of Virginia deer at monthly intervals throughout the coming year. The status of the parathyroid gland will be examined in these specimens, and further discussion of the problem will be deferred until after this more extensive investigation has been completed.

The specimens of Virginia deer were obtained through the courtesy of the trustees of Naushon Island.

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GROWTH OF VITAMIN DEFICIENT RATS TREATED WITH THYMOCRESCIN[1]

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ONE OF US (W.O.N., 1) has recently reviewed the literature on thymic physiology, but a few historical points need emphasis here. In 1929 Asher and Ratti (2) reported growth promoting effects of aqueous extracts of calf thymus. They found that young rats, maintained in darkness on McCollum's rachitogenic diet and injected with thymic extract, continued to grow and showed no clinical or histological evidence of rachitis.

Asher (3) has reviewed the subsequent work of his group and set forth the procedure for the preparation of the most potent extracts. These have marked growth promoting and gonadotropic effects in dosages as low as 1 mg./day on animals on deficient diets. The material is characterized as a protein and lipid-free polypeptide.

MATERIAL AND METHODS

Following Asher's procedure (3), 150 kg. of fresh calf thymus were processed, yielding 263 gm. of dry thymocrescin.[2] This was dissolved in normal saline solution in concentrations of 5 and 10 mg./cc., in lots sufficient for 2 or 3 days, and kept under refrigeration. Daily subcutaneous injections of 1 cc. were initiated at weaning. A sterile solution of 20 mg./cc. was employed in the immunization studies.

The rats used were from our standard hooded stock, and were weaned onto one of two diets: *a*), a synthetic thiamin-deficient diet (Parke, Davis & Co.'s No. 98067 —generously supplied by Dr. Emmitt) approved by Asher; or *b*), a pure diet of horse meat (kindly furnished by Chappel Bros., Inc.) suggested by Asher.

The immunization experiments were made upon 4 male and 4 female stock guinea pigs (334 to 470 gm.), maintained on Purina Rabbit Ration and added lettuce.

EXPERIMENTAL

Chemical analyses of extracts. Table 1 compares our qualitative chemical tests with those of Asher, who (personal communication) says of them: "Your chemical tests seem to me very satisfactory and they speak in favor of the probable purity of your substance." Asher and his coworkers have not reported on nitrogen content, ash, iron, or sulfhydryl groups. Three of our preparations gave respective nitrogen values of 16.55, 15.13, and 16.77%. One preparation yielded 1.99% ash, and no iron. The nitroprusside test for sulfhydryl groups was negative on several preparations.

Injection experiments. Figure 1 shows the growth of 18 male rats on the thiamin-deficient diet. No acceleration of growth was obtained on either dosage of thymo-

[1] This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

[2] The glands were purchased from Swift & Co., who froze them immediately after killing and shipped them to Parke, Davis & Co. We are grateful to Drs. Oliver Kamm and D. A. McGinty of Parke, Davis & Co. for handling the preliminary acetone and ether extractions.

crescin. The autopsy data for these animals (at 70 days) are given in table 2, which shows that the treated animals weighed less than the controls, although bodydimensions were essentially the same in all series.

The weights of testicle, kidney, heart, adrenal, thyroid and pituitary were about

TABLE 1. CHEMICAL ANALYSES OF THYMOCRESCIN

	Asher's preparations	Our preparations
Labile sulphur	positive	positive
Ninhydrin	positive	positive
Biuret	positive	positive
Hopkins-Cole	negative	faint positive[1]
Xanthoproteic	questionable	faint positive
Millon	positive	positive
Molish	positive	positive

[1] The Hopkins-Cole reaction in our preparation was negative when the boiling in acid solution was extended to 30 minutes instead of the 10 minutes which was found to precipitate all of the heat coagulable proteins.

the same in the controls and the 2 experimental series. The large spreads in thymic and prostatic weights which occur in normal animals would account for the differences observed in the weights of these organs. The apparently greater weight of the full seminal vesicles in the treated animals was probably due to the fortuitous presence of additional secretion at the time of autopsy, since the weights of empty seminal vesicles agree closely among the three series.

Figure 2 shows the growth of 8 rats on the horse-meat diet. It is apparent that no

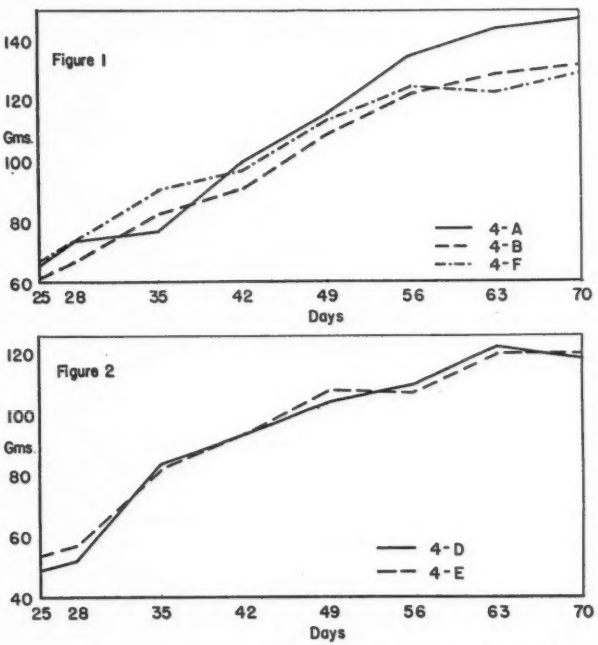

Fig. 1. MALE RATS ON THIAMIN-DEFICIENT DIET #98057. 4-A: control; 4-B: 5 mg. thymocrescin/day; 4-F: 10 mg. thymocrescin/day. Fig. 2. RATS ON HORSE-MEAT DIET; 4-D. control; 4-E: 5 mg. thymocrescin/day.

acceleration in the rate of growth was induced by treatment with thymocrescin.

Immunization experiment. Each guinea pig was given a single intraperitoneal injection of 20 mg. thymocrescin. Four animals given 20, 30 or 40 mg. intravenously after 3 weeks showed no evidence of anaphylaxis. Two others tested similarly 4 weeks after the original injection were negative as well, as were also the remaining 2 animals tested by intradermal injections five weeks after their original injection.

TABLE 2. AUTOPSY DATA AT 70 DAYS FOR 18 MALE RATS ON THIAMIN-DEFICIENT DIET NO. 98067

	Weight	Body length	Tail length	Span, fore limbs	Span, hind limbs
Control	152 gm.	17.8 cm.	15.8 cm.	15.0 cm.	19.8 cm.
Thymocrescin, 5 mg.	132 gm.	16.6 cm.	15.3 cm.	14.9 cm.	19.3 cm.
Thymocrescin, 10 mg.	137 gm.	18.0 cm.	16.2 cm.	15.3 cm.	19.4 cm.

	Rt. Testis	Rt. kidney	Thymus	Seminal Vesicles Full	Seminal Vesicles Empty	Heart	Prostate	Adrenal	Thyroid	Pituitary
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Control	1,182	950	156	105	63	560	89	31	11	4.7
Thymocrescin, 5 mg.	1,067	944	120	112	64	544	104	33	12	3.6
Thymocrescin, 10 mg.	1,126	853	103	153	55	584	113	31	13	5.0

DISCUSSION

Extracts of calf thymus glands prepared by the method of Asher and his coworkers agreed closely with their extract as to chemical analyses. However, despite the use of diets either suggested or approved by Asher, and careful duplication of all procedures none of the physiological activity reported by the Swiss workers could be demonstrated.

Since it seemed strange that the Asher group should characterize thymocrescin as being free of proteins despite the fact that it gives the common tests for proteins, the immunization experiment was designed to see if thymocrescin acted immunologically as a typical protein. In our hands egg albumin, a typical protein, produced anaphylaxis under the stated conditions. We were unable to demonstrate immunologically that thymocrescin acted like a typical protein. However, the experiment is not conclusive in that regard since it is exceedingly difficult to immunize with some proteins (4).

SUMMARY

A purified aqueous extract, thymocrescin, was prepared from calves' thymus glands. The preparation agreed closely with published chemical analyses. No growth promoting or gonadotropic effect could be demonstrated upon 18 rats on a thiamin-deficient diet or upon 8 rats on a horse-meat diet. Attempts to immunize 8 young guinea pigs to thymocrescin were unsuccessful.

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AN ATTEMPT TO PRODUCE INCREASED SUSCEPTIBILITY TO RENAL ISCHEMIA IN RABBITS BY MEANS OF ENDOCRINE PREPARATIONS

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THE DELETERIOUS EFFECT of pregnancy on the course and prognosis of chronic nephritis has long been noted by obstetricians (1, 2, 3). Acute nephritis coincident with pregnancy is apparently so rare that no statistics are available. We have been able to demonstrate that dogs and rabbits are made much more susceptible to the effects of acute renal ischemia by the pregnancy state (4).

The isolation of a pressor substance (5, 6) from the kidney together with the failure to ameliorate the hypertension of renal ischemia by nerve (7) and spinal cord sections (8) and renal transplantation (9) makes it likely that this pressor substance is the fundamental agent in producing this type of hypertension. The altered reaction of the pregnant animal to the reduction of renal blood flow makes it seem probable that the vasospastic properties of this pressor substance (renin) have been enhanced by some agent which is found exclusively or in greater abundance in the pregnant animal.

Greene (10) has shown that the fetus and placenta can be ruled out as the causative agent of the spontaneous eclampsia of the rabbit and has recently (11) been able to reproduce accurately this clinical and pathological picture with the injection of saline extracts of the anterior lobe of the pituitary in the pregnant animal. Page (12) has pointed out that the fetus can be ruled out as a factor in the pathogenesis of the human disease as evidenced by the high incidence of toxemia of pregnancy in patients who have hydatidiform mole. Dietel (13) reported the production of liver lesions in experimental animals by the injection of extracts of the intermediate lobe of the pituitary. Byrom (14) has demonstrated lesions of the liver and kidney of rats which have received repeated injections of posterior pituitary extracts.

It seems possible that the agent or agents which convert the renal ischemia syndrome from a benign hypertensive state in the non-pregnant animal to a malignant fatal disease in the pregnant animal may be hormonal in character. The following experiments are designed to determine if one or more of the commonly used glandular extracts are able to change the response of the non-pregnant rabbit to renal ischemia.

MATERIALS AND METHODS

Fourteen female rabbits of 2 to 3 kg. weight were used as the experimental animals. They were segregated for approximately 3 weeks before use to rule out pregnancy and pseudopregnancy. Feed consisted of dry oats and hay daily with supplementary feedings of lettuce and carrots 3 times weekly. Water was given ad libitum. All animals were considered to be in good physical condition and showed no pathologic changes of the viscera at laparotomy.

The animals were divided into 7 groups of 2 animals each. Blood pressure read-

ings (15) were made daily and examination of the urine for specific gravity, albumin, and sediment was made bi-weekly on all animals for periods of 1 to 3 weeks. Both animals in each group were injected with the same hormone at 8-hour intervals by the intramuscular route for 1 day preceding the reduction of the renal blood flow and for 5 days following this procedure.

To the animals of group 1 the ovarian hormone was given; of group 2 the hormone of the corpus luteum; of group 3 the adrenal cortical hormone; and of group 4 a saline extract of the anterior lobe of the pituitary (Lilly). The gonadotropic hormone from pregnancy urine was given to the animals of group 5; the oxytocic factor of the posterior pituitary to the animals of group 6 and the vasopressor factor of the posterior lobe of the pituitary to those of group 7.

The hormone under investigation was given for 1 day and following this the aorta was constricted by means of a silver wire loop proximal to the points of origin of the

TABLE 1. THE EFFECT ON URINE, BLOOD PRESSURE AND CLINICAL CONDITION OF CONSTRICTING THE AORTA PROXIMAL TO THE POINTS OF ORIGIN OF THE RENAL ARTERIES TO ½ OF THE ORIGINAL CROSS-SECTIONAL AREA

Group	Rabbit No.	Medication	Alb.	Days	Blood pressure	Condition	Biopsy or autopsy findings
Control 1	A	None	0	0	+24	Good	No biopsy.
	B	None	0	0	+30	Good	No biopsy.
Control 2	A	Pregnant	+++ (RBC)	3	− 2	Poor	Delivered.
	B	Pregnant	++++ (RBC)	5	−25	Died	Necrosis of kidney, fatty change of liver.

renal arteries to approximately one-half of its original cross-sectional area (16). The injection of the hormone was continued for the next 5 days and daily blood pressure estimations were made, urine studies made, and close observation of the clinical condition recorded. If no significant change was noted in the animal following 10 days of such observation the same studies were continued at less frequent intervals.

To demonstrate the striking differences between the pregnant and the non-pregnant animal in their reaction to equivalent aortic constriction, and thus to present a basis for comparison of the results produced by injection of hormone preparations and by pregnancy, 2 non-pregnant and 2 pregnant female rabbits of the same weight and condition as those of the experimental group were used. The non-pregnant animals had been segregated for at least 3 weeks; the pregnant animals had been observed for approximately 3 weeks and were 15 to 20 days in the period of gestation. Aortic constriction was carried out in these animals to a degree equivalent to that of the experimental animals, and the same postoperative period of observation was followed.

Following the death of any animal a complete autopsy was made within 4 hours and the cause of death ascertained if possible. Sections of all organs were taken, fixed in Helley's modification of Zenker's solution, embedded in paraffin, and stained with hematoxylin and eosin.

A liver biopsy was taken on any animal in which the clinical course, urine, or blood pressure studies seemed to indicate more evidence of renal damage or change in general condition than was noted in the non-pregnant control animals. The biopsy was usually made on the day following the last hormone injection.

RESULTS

Table 1 summarizes the effect on blood pressure, urine and general condition of

constriction of the aorta proximal to the points of origin of the renal vessels in 2 pregnant and 2 non-pregnant rabbits. It is noted that this procedure in the pregnant animal is followed by a progressive downhill course, albuminuria, hematuria and a slight to moderate depression of the blood pressure, while in the non-pregnant animal only a moderate hypertension is produced with no evidence of renal injury or of toxic effects.

Table 2 demonstrates the effect of the same degree of aortic constriction on animals receiving the various hormone preparations. All of these preparations seemed to produce a noticeable but probably not significant difference in the animals' responses when compared with the relatively characteristic response of the non-pregnant control animal.

TABLE 2. EFFECT OF CONSTRICTION OF THE AORTA PROXIMAL TO THE POINTS OF ORIGIN OF THE RENAL ARTERIES TO ½ OF THE ORIGINAL CROSS-SECTIONAL AREA ON RABBITS RECEIVING THE SPECIFIED AMOUNTS OF THE LISTED HORMONE PREPARATIONS

Group	Rabbit No.	Medication	Daily dosage	Urine Alb.	Urine Days	Blood pressure	Condition	Biopsy or autopsy findings
1	22 A	Amniotin	7,200 I.U.	+	8	+32	Good	No biopsy.
	33 B	Amniotin	14,400 I.U.	+	11	+40	Good	No biopsy.
2	21 A	Proluton	3 mg.	VFT	4	+22	Good	No biopsy.
	28 B	Proluton	6 mg.	+	1	+16	Good	No biopsy.
3	20 A	Eschatin	3 cc.	VFT	1	+ 4	Good	No biopsy.
	32 B	Eschatin	6 cc.	VFT	1	−15	Good	Liver biopsy normal.
4	24 A	Ant. pit.	3 cc.	+	3	+50	Good	No biopsy.
	34 B	Ant. pit.	6 cc.	0	0	+ 9	Good	No biopsy.
5	25 A	Follutein	500 R.U.	0	0	−12	Good	No biopsy.
	26 B	Follutein	1,000 R.U.	++	6	+ 6	Good	Liver biopsy normal.
6	30 A	Pitocin	30 O.U.[1]	Tr.	1	+ 4	Good	No biopsy.
	31 B	Pitocin	60 O.U.	+	2	+21	Good	No biopsy.

[1] Oxytocic units.

The symbols under "Urine" represent:

Albumin Tr.: No ppt. after heating, slight clouding after acid.
+: Granular ppt. after heating.
++: Heavy granular ppt. after heating.
+++: Heavy flocculate ppt. after heating and acid.
++++: Boils solid on heating.
RBC Blood in the urine. Benzidine or microscopic.
Days The number of days the albuminuria persists.

Under "Blood pressure" the figures represent the average blood pressure for the periods of observation after aortic constriction as compared with the control observations.

The animals receiving amniotin and proluton showed no significant changes in the blood pressure, urine, or general condition, and no biopsies were made.

The smaller intramuscular dosages of follutein were accompanied by no significant changes although the larger ones (1000 I.U. daily) were followed by moderate albuminuria, minimal depression of the blood pressure, and slight loss of gloss on the fur. Because of these suggestive changes 2 additional animals were given 500 I.U. and 1000 I.U. daily in 3 equal doses by the intravenous route. No significant changes in the blood pressure, urine, or general condition were noted in these animals. Liver biopsies made on both animals receiving the hormone intravenously and on the animal to which the larger intramuscular dosage had been given showed no evidence of injury.

Both animals receiving eschatin experienced slight albuminuria and a minimal depression of the blood pressure. The general condition of the animals seemed good and a liver biopsy on the animal to which 6 cc. of the hormone had been given daily showed no injury.

Anterior pituitary solution and pitocin caused no noticeable variations in blood pressure, urine, or clinical condition, and no biopsies were made on these animals.

Because of the significant changes noted in the rabbits to which pitressin was administered, additional animals were tested. Table 3 shows the effect of this preparation on representative animals.

TABLE 3. THE EFFECT OF THE CONSTRICTION OF THE AORTA PROXIMAL TO THE POINTS OF ORIGIN OF THE RENAL ARTERIES TO ½ OF THE ORIGINAL CROSS-SECTIONAL AREA IN ANIMALS RECEIVING VARYING DAILY DOSAGES OF PITRESSIN. (Each cc. of pitressin contains 20 pressor units.)

Group	Rabbit No.	Daily dosage	Urine Alb.	Days	Blood pressure	Condition	Biopsy or autopsy findings
		cc.					
Pitressin A	23	3	++	7	− 2	Fair	No biopsy.
	41	3	Tr.	1	− 4	Good	Killed. Hemorrhage in adrenals and stomach.
Pitressin B	35	4.5	+	4	−22	Fair	Killed. No lesions.
	36	4.5	++	3	−22	Died	Necrosis of adrenals and stomach.
Pitressin C	27	6	++++ (RBC)	2	−80	Died	Necrosis of liver, kidneys, adrenals, and stomach.
	54	6	++	2	Too low	Fair	Necrosis of adrenals and stomach.

Following aortic constriction to a degree equivalent to that carried out on the preceding animals, pitressin was given to 8 animals 3 times daily by the intramuscular route. Each of 2 animals received 3 cc. and 4.5 cc. daily; 4 animals were given 6 cc. daily.

All these animals showed albuminuria, depression of the blood pressure and progressive weakness. All save one animal receiving 6 cc. daily developed mild hematuria, severe albuminuria and died. One of the rabbits receiving 4.5 cc. daily also succumbed while the other was killed one day following the last injection. Both of the animals which received 3 cc. of pitressin daily, survived and one was killed on the day following the last injection.

Of the 7 animals examined postmortem, all save one, the rabbit which received 4.5 cc. daily and did not die, showed adrenal and gastric lesions. Five showed severe renal injury, 4 had necroses of the liver, and 1 showed severe injury to the myocardium.

Eight animals in which the aorta had been constricted to approximately ⅓ of the original cross-sectional area below the points of origin of the renal arteries were used as control group 1. Six of these animals received 6 cc. of pitressin daily and the remaining two 9 cc. of the hormone each day. Table 4 shows the effect of this procedure on 2 representative animals of this group and 2 of the following group (control group 2).

It seemed possible that vascular spasm produced by pitressin in the kidney of the control animals might be of such severity as to produce a renal ischemia similar in degree to that obtained by clamping the aorta or the renal arteries, and so might produce the same effect as that sought in animals given pitressin following aortic con-

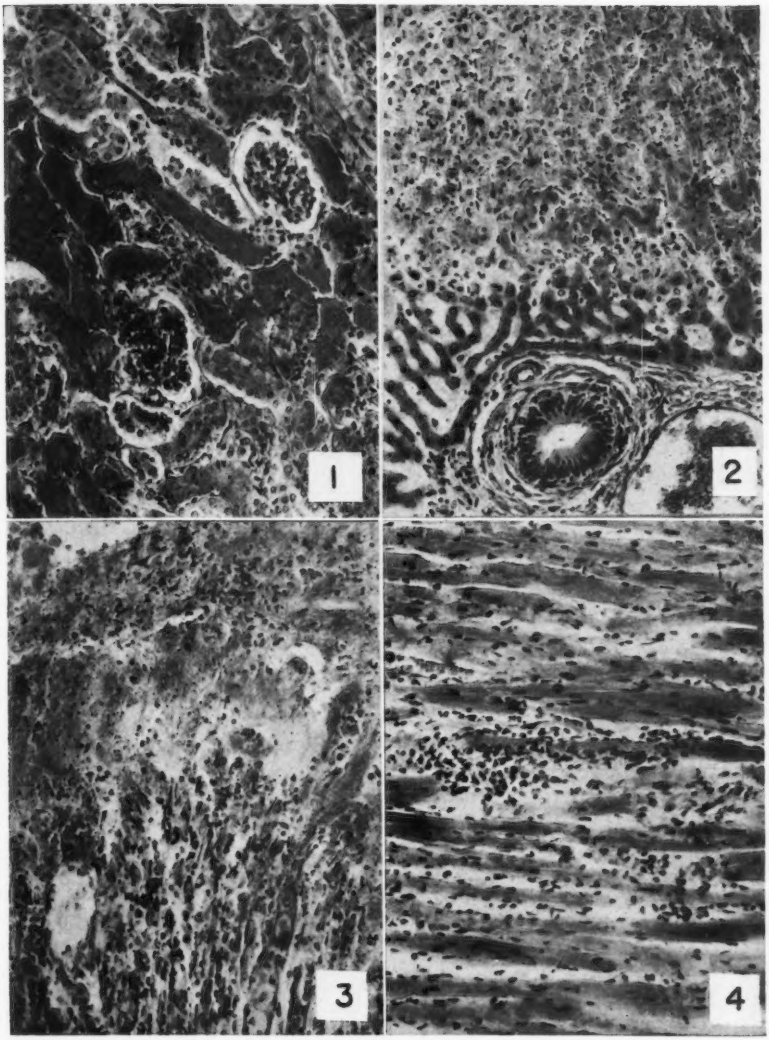

Fig. 1. SECTION SHOWING INJURED AND HYALINIZED CONVOLUTED TUBULES. Note the lack of injury to the glomeruli and to the remaining convoluted tubules. Fig. 2. SECTION OF LIVER SHOWING A LARGE AREA OF MID-ZONAL NECROSIS. Note the normal appearance of the liver tissue surrounding the portal field. Fig. 3. SECTION OF GASTRIC MUCOSA SHOWING NECROSIS OF THE PERIPHERAL PORTION AND FORMATION OF A DIPHTHERITIC MEMBRANE. Fig. 4. SECTION OF HEART SHOWING MYOFIBRIL DEGENERATION AND FRAGMENTATION WITH CELLULAR INFILTRATION. (Photographs by Alberta Kuder.)

striction above the renal arteries. Therefore, 4 rabbits were nephrectomized and then injected with 6 cc. of pitressin daily in the same manner as the experimental animals.

These rabbits responded in a similar manner clinically and presented the same type and extent of pathologic lesions as the animals in which the kidneys were intact.

When lesions were found in animals receiving pitressin, they were identical irrespective of the presence of the kidney or the position of the clamp on the aorta in relation to the renal arteries, and the following descriptions apply to all animals receiving pitressin.

The gross appearance of the kidney was usually not remarkable save for moderate 'cloudiness' of the parenchymatous tissue. Rarely a small 'flame-shaped' hemorrhage extending toward the capsule through the cortex was seen. Such hemorrhages were found even more rarely in the medullary portion. Occasionally small, pale yellow, bloodless areas in the cortex were observed.

TABLE 4. THE EFFECT OF PITRESSIN INJECTIONS UPON CONTROL ANIMALS IN WHICH AORTIC CONSTRICTION TO ½ OF THE ORIGINAL CROSS-SECTIONAL AREA WAS CARRIED OUT DISTAL TO THE POINTS OF ORIGIN OF THE RENAL ARTERIES. GROUP D REPRESENTS NORMAL ANIMALS; GROUP E REPRESENTS ANIMALS FOLLOWING TOTAL NEPHRECTOMY. (Each cc. of pitressin contains 20 pressor units.)

Group	Rabbit No.	Daily dosage	Urine Alb.	Urine Days	Blood pressure	Condition	Biopsy or autopsy findings
		cc.					
Pitressin	40	6	++	4	−20	Died	Necrosis of liver, stomach, and kidneys.
D	62	6	++	3	Too low	Died	Necrosis of liver and kidneys.
Pitressin	63	6	—	—	−20	Died	Necrosis of liver and stomach.
E	64	6	—	—	Too low	Died	Necrosis of liver and stomach.

Microscopically the glomeruli were usually normal. Occasionally a convoluted tubule which contained a few hyalinized cells with pyknotic nuclei or a moderate amount of albumin in the lumen were seen. The hemorrhages seen grossly were small extravasations of blood into the interstitial tissue, the parenchymatous tissue in that area showing a minimal amount of injury such as hyalinization or hydropic changes of the tubule cells or small collections of edema around the tubules in these areas. In the more seriously injured organs the convoluted tubules were converted into hyalinized semblances of the former tissue, the nuclei were absent, and the lumina obliterated. The glomeruli in such areas were usually free from obvious signs of injury and no arteriolar changes of significance could be made out. Figure 1 demonstrates severe renal injury of this type.

The liver of these animals frequently showed some cloudy swelling, a slightly stretched capsule, and in small and irregularly placed areas, whitish-gray tissue which seemed to conform to the pattern of the lobule. Occasionally there could be found just beneath the capsule an area of this type of discoloration which occupied several square millimeters and which extended into the liver tissue for a space of 1 to 2 millimeters.

Microscopically these areas of necrosis were noted to be focal in distribution and were usually seen in the mid-zonal areas of the liver lobule. They were usually sharply outlined and began in the peripheral one-third of the lobule, extending practically to the central vein. The liver cells in these areas were confluent masses of hyalinized material in which the nuclei were pyknotic or fragmented and the sinusoids partially or completely obliterated. In the larger areas several lobules were injured in this same manner, but even in these areas it was unusual to see cells immediately surrounding the portal field in poor condition (fig. 2).

The injured adrenal glands were characteristic in the gross because of multiple 'flame-shaped' hemorrhages which extended from the middle one-third of the cortex almost out to the capsule but rarely extended into the medullary portion.

Microscopically the less severely injured glands showed small, round, circumscribed areas of necrosis in the cortex which were characterized by hyalinization of the cells, extreme fragmentation of the nuclei, and a considerable infiltration of blood into and around the areas of injury. The more severely injured organs showed the same type of picture on a more extensive scale, and in some of these glands the only living adrenal tissue was a thin layer lying directly beneath the capsule.

The gastric mucosa usually presented a very striking picture. Minimal injury consisted of numerous petechial hemorrhages into the mucosa. Severe injury was characterized by ulceration of the mucosa and in some places a diphtheritic membrane which might occupy several square centimeters of gastric surface was seen.

Microscopically the petechial hemorrhages occurred in the interstitial tissue between the crypts of Lieberkuhn and varied considerably in amounts of extravasated blood. The ulcerated areas showed complete loss of the distal two-thirds of the gastric mucosa with the ulcer base being filled in with hemorrhage, round cell and polymorphonuclear leukocytic infiltration. The membrane was characterized by a massive collection of fibrin, polymorphonuclear leukocytes and red blood cells, attached firmly to the underlying mucosa which showed considerable numbers of hyalinized cells, leukocytes and nuclear fragments (fig. 3).

The single instance of myocardial injury noted was grossly represented by an area of yellow, pale tissue which extended over the lower third of the left ventricle down to the apex from the endocardial to the epicardial surfaces.

Microscopically it showed considerable fragmentation of the myofibrils, a moderate amount of round cell and wandering cell infiltration, and a rare leukocyte. Hemorrhage was noticeable but not profuse (fig. 4). The lungs of most of these animals showed marked pulmonary edema. The remainder of the organs showed no constant or significant lesions.

DISCUSSION

We have attempted to find a hormone which will render the response of the non-pregnant rabbit to renal ischemia similar to that seen in the pregnant animal when subjected to this procedure.

Of the preparations tested, only pitressin seems to fulfill these requirements. This is in agreement with work done by Williams, Diaz, Burch and Harrison (17), who were unable to demonstrate increased pressor response of the rat to renin when injected with several hormone preparations.

We have not, however, been able to show that the effect of pitressin is synergistic, or even additive, to the effect of renal ischemia. This is surprising in that both are vasospastic agents and that either very severe renal ischemia alone (15) or pitressin alone will produce the same type of clinical syndrome and pathologic lesion even in the non-pregnant animal.

It also seems clear that pitressin does not exert or enhance its effect by the liberation of renin through renal vascular spasm.

Since the original investigation of Anselmino and Hoffmann (18) much work has been done in an effort to demonstrate appreciable amounts of the antidiuretic substance in the blood of patients with toxemia of pregnancy. Although careful work (19) has failed to demonstrate its presence in the blood, it has been reported in consistently elevated amounts in the urine of such patients (20).

It seems probable that none of the preparations tested are responsible for transforming the renal ischemia syndrome in the animal, and possibly the toxemia of pregnancy syndrome in the human being, from a benign (and usually sub-clinical) nephritis into a malignant vascular disease.

SUMMARY

Rabbits receiving varying dosages of several commercial hormone preparations were subjected to renal ischemia by moderate equivalent constriction of the aortas proximal to the points of origin of the renal arteries.

The following hormones were given 3 times daily by the intramuscular route: amniotin in daily dosages of 7200 I.U. and 14,400 I.U.; proluton in daily dosages of 3 mg. and 6 mg.; eschatin in doses of 3 cc. and 6 cc. daily; saline extracts of anterior pituitary in daily doses of 3 cc. and 6 cc.; follutein in daily dosages of 500 R.U. and 1000 R.U.; and pitocin, 30 O.U. and 60 O.U. daily. None of the animals showed significant variations in urine findings, blood pressure levels, or histologic changes in the liver and kidney in comparison with animals in which equivalent aortic constriction had been performed.

Animals receiving 60 to 120 P.U. of pitressin showed albuminuria, depression of the blood pressure, and lesions of the kidney, liver, adrenal, myocardium and gastric mucosa when subjected to comparable renal ischemia.

Using approximately the same dosages of pitressin, similar blood pressure, urine and pathologic changes were produced in control animals in which aortic constriction was performed below the points of origin of the renal arteries.

No synergistic effect of these preparations on the course of animals subjected to renal ischemia could be demonstrated.

The authors wish to take this opportunity to thank Dr. E. A. Sharp and Parke, Davis & Company for generous supplies of pitressin, pitocin, and eschatin used; Dr. Max Gilbert and the Schering Corporation for the proluton donated; Mr. Joseph A. Loughrey and Dr. Raymond M. Rice of Eli Lilly & Company for the anterior pituitary solution; and Mr. Charles L. Morris, Mr. Clifford Halstead and E. R. Squibb & Sons for supplies of amniotin and follutein.

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CORTIN AND THE Na FACTOR OF THE ADRENAL[1]

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THE USUAL EXTRACT of the whole adrenal or of the adrenal cortex will prevent the loss of sodium which occurs in adrenal insufficiency. It will also cause retention of sodium in the normal mammal (1). Such an extract can be divided into two fractions, one responsible for sodium retention and the other capable of maintaining the health of adrenalectomized animals without influence on sodium retention. The substance primarily responsible for sodium retention has been designated by us as the Na factor. The substance in the second fraction, responsible for maintenance of adrenalectomized animals but without influence on Na, we have tentatively designated as cortin (2).

The separation of these two fractions was impossible until a method had been devised for the detection and assay of the Na factor. This is so essential to the present study that we shall present it in detail.

Normal adult female dogs weighing between 6 and 13 kg. were used. These had been under observation in the laboratory for several weeks. Animals which consumed their ration within 15 minutes after feeding were selected because it was necessary to have absolute control of the time of the electrolyte intake. Only animals that were easily catheterized were employed. All animals were immunized against distemper and freed from worms. They were fed a constant diet consisting of a mixture of ground beef heart, Purina chow and 2 gm. of added NaCl once daily. The feeding was at 11 P.M., which was 9 hours before the beginning of a test period. Earlier feeding permitted the elimination of the excess electrolytes before the test period so that little remained for the demonstration of Na retention. They were allowed as much water as they desired, except on test days. During a test the animal was kept in a metabolism cage. The test was started at 8 A.M. after the bladder had been emptied by catheterization. The extract was injected subcutaneously and 100 cc. of water was administered by stomach tube. The subcutaneous path was necessary to avoid development of refractoriness which occurs with repeated intravenous injection (3). The urine was collected by catheterization at the end of 6 hours because it was found that longer periods were unnecessary, the maximum effect being attained in from 3 to 6 hours. Toluol was used to preserve the urine. The room temperature was maintained at about 23° C. during the whole period of study. (In hot weather it is impossible to perform the test.) The amounts of sodium, potassium, ammonia and chloride as well as the urinary volume during the 6-hour period, were compared with corresponding determinations during similar periods on other days in which no injection was made or only an equal volume of an inert solution injected. The methods used for electrolyte determination are described elsewhere (3). The Na factor produced little or no change in the excretion of total base, increase in the output of potassium and ammonia compensating for sodium retention. Urine volume

[1] Aided by a grant from the Rockefeller Foundation

also changed little. Thus it was possible to show a specific retention of Na (3). Only the Na values are given in this report because the other values were used merely to show that the effects were not due to general retention. Animals were not used for assay until they maintained a constant weight on a standard diet and control periods indicated that electrolyte balance had been established. One or more control determinations were made between assays to ascertain whether or not the animal had returned to normal, extract tests not being repeated until this was so.

Typical values are given in table 1. It will be observed that repeated tests of the same dose gave results that check fairly well. It will also be noted that when the retention became very high the values were not proportionate to the dose, moderate ranges being more accurate.

Twenty-four-hour periods are not more accurate than the carefully controlled 6-hour period. This is shown by repeated tests in the same dog (3*M*). Two injections of the same dosage and lot of extract, 12 hours apart, were followed by retentions for the 24 hours as follows: 25, 29, 27.5, 19 and 26.4% (compare with values in table 1).

TABLE 1. COMPARISON OF Na RETENTION WITH THE SAME DOSE AND WITH DOSES OF INCREASING MAGNITUDE DURING A 6-HOUR PERIOD

Animal	Preparation	Na retention, %
Dog 33	10 cc. Extract No. 1	47
Dog 33	10 cc. Extract No. 1	41
Dog 32	10 cc. Extract No. 1	48
Dog 32	10 cc. Extract No. 1	47
Dog 32	4 mg. corticosterone	29
Dog 32	4 mg. corticosterone	27
Dog 31	0.5 mg. desoxycorticosterone acetate	17
Dog 31	1.0 mg. desoxycorticosterone acetate	32
Dog 31	2.0 mg. desoxycorticosterone acetate	56
Dog 31	4.0 mg. desoxycorticosterone acetate	68

Within certain limits the effect of the Na factor is independent of the size of the animal. Assay of the Na factor should be based on a Na retention higher than the daily variation of control periods and lower than the region of decreased response. After a study of a large number of determinations we have chosen the quantity of hormone which will cause a 30% retention as a basis for assay. This quantity is so large that we have divided it by 10 for the unit, otherwise, significant values would have to be indicated as fractions. Therefore our unit for the Na factor is one-tenth of the quantity which will cause a 30% retention of Na over a 6-hour period in a normal dog (5 to 15 kg.).

The assay of cortin is based on the maintenance of the appetite and health of adrenalectomized cats (4). When Na factor is proven to be absent by the dog test, cortin assay gives true values, but in the presence of the Na factor, cortin assay is likely to give high values. Although the Na factor alone does not maintain the appetite at the normal level, it may act synergistically so that somewhat less cortin is required in its presence.

Adrenalectomized cats in excellent condition were used for assay. They were kept at fairly constant temperature (27 to 29° C). and were fed the same amount of food (beef heart, canned salmon and milk) daily together with vitamin supplement every few days (4). Only those animals with a regular appetite were employed, the

optimum amount of food having been determined by many trials before adrenalectomy. An experienced person familiar with the individual animals should conduct the assay. He readily notices changes in behavior which might be overlooked by the casual observer. Increase in eating time frequently precedes reduction of food intake.

The extract was injected subcutaneously twice daily, the sum of the injections being the daily dose. The quantity of extract was reduced every fourth day (usually by 50%) until there was a reduction in food intake. The last dose above that in use when the appetite first diminished, was taken as the maintenance dose or end point. The cat unit for cortin is the minimum amount of hormone required daily to maintain the appetite and health of an adult adrenalectomized cat (without NaCl supplement).

Preparation of Cortin Free from Na Factor

The method employed for the removal of the Na factor from cortin involves a long series of interchanges between water and ethyl ether. It is designed to produce a water extract of cortin completely free from the Na factor without subjecting the material to the hazards of drying (Flow Sheet 1).

The tissue is extracted twice with ethyl alcohol. The extracts are combined and reduced in volume by distillation. The resulting aqueous alcohol residue is extracted with ethyl ether. Cortin, by passing into the ether, leaves behind part of the Na factor and much of the water-soluble impurities which are discarded with the aqueous solution. The ether is removed by distillation and alcohol is added to the residual solution containing the cortin to make a concentration of 70% alcohol. Chilling to −20° C. deposits a precipitate which may be discarded by filtration. This precipitate contains very small amounts of Na factor and cortin together with a large amount of inert material. After the alcohol is removed the aqueous extract is frozen then thawed. A second precipitate, which forms, is discarded by filtration at a temperature just above freezing. These steps are necessary because among the precipitated impurities are materials which, if allowed to remain, cause emulsification during subsequent extractions. To remove the remaining Na factor a series of interchanges between water and ethyl ether is employed. The Na factor is but slightly soluble in water but readily soluble in ethyl alcohol above 5% concentration. The aqueous solution left after filtration of the thawed material is extracted 8 times with equal volumes of ether. The ether extracts cortin, Na factor and alcohol. The aqueous solution is discarded. Removal of the ether by distillation carries off a part of the alcohol. Water is added to make up to the original volume. This precipitates the Na factor, which is removed. The whole process, ether extraction, distillation and precipitation, is repeated until no Na factor remains.

The part played by each step in the foregoing procedure may be illustrated by the following determinations. After chilling the aqueous concentrate from the 70% alcohol solution, the first series of ether extractions contained 13 U of Na factor per kg. of tissue, the second series contained 5 U per kg., the third series 0.8 U per kg. and the fourth series contained no detectible amount.

It has been impossible to recover the Na factor when the separation of the two factors has been made by ether-water interchange.

In the long process of removing the Na factor some cortin is lost, so that the final yield is perhaps only one-half to two-thirds of what might be expected from a less complicated procedure. There is, however, an increase in purity of the product over that at the start, the potency being 4 to 8 C.U. instead of 3 to 4 C.U. per mg. Epinephrin was absent according to the Folin, Cannon and Denis test (5).

Preparation of Na factor. Acetone extraction of adrenal tissue gives a high yield

FLOW SHEET 1

SEPARATION OF Na FACTOR FROM CORTIN

12 kg. of adrenal tissue extracted with 24 l. of 95% ethyl alcohol.

First alcohol extract.

Tissue residue extracted with 8 l. of 80% alcohol.

Second alcohol extract (contained 8 C.U./kg. of cortin, 2 U/kg. of Na factor).

Combined extracts reduced to 2 l. vol.*

Residue discarded contained 3 C.U./kg. cortin, 35 U/kg. Na factor.

Concentrate extracted 6 times with equal volumes of ethyl ether.

Ether extract. After removal of ether, alcohol added to 70% conc.; vol. 1.5 l. Alcohol solution chilled to −20° C. and filtered.

Aqueous solution discarded; cortin, 0; Na factor, 2 U/kg.

Alcohol removed from filtrate. Aqueous concentrate frozen then thawed and filtered; vol. 500 cc.

Precipitate discarded; cortin, 3.6 C.U./kg.; Na factor, 4 U/kg.

Aqueous solution extracted 8 times with equal volumes of ethyl ether.

Precipitate discarded; cortin, 0; Na factor, 0.

Ether solution. Water added and ether removed by distillation, final vol. 250 cc.

Aqueous solution discarded.

Filtered

Aqueous solution extracted 8 times with equal volumes of ethyl ether.

Precipitate discarded.

Ether solution. Series of ether-water interchanges and filtration repeated until Na factor is removed.

Aqueous solution discarded; vol. 200 cc.

Ether solution. Water added then ether removed by distillation, ppt. discarded.

Aqueous solution discarded.

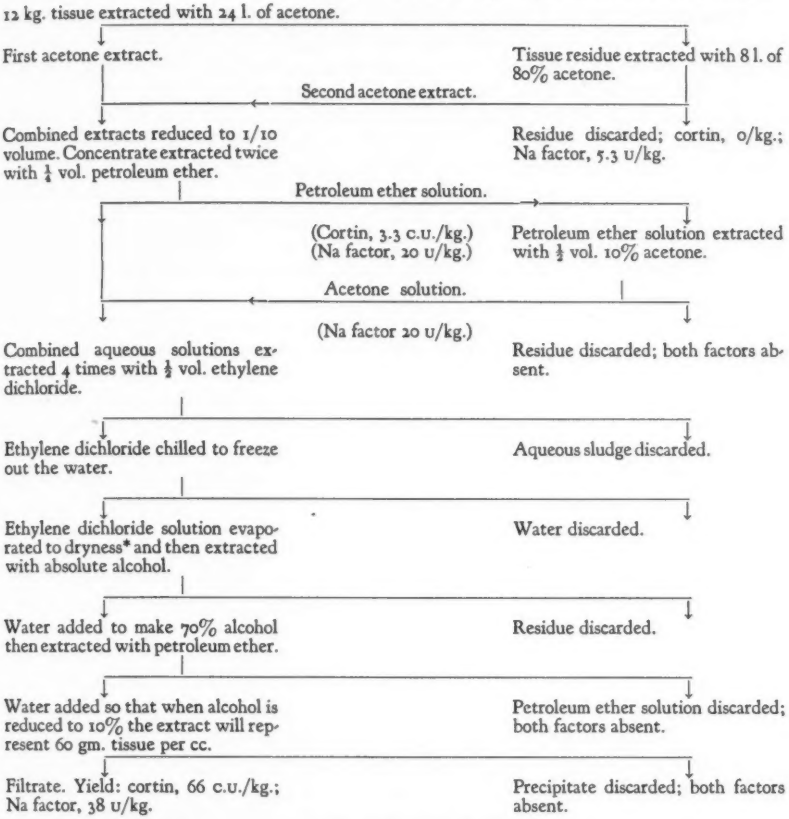
Final aqueous extract, vol. 200 cc. Yield, 32 to 40 C.U. cortin/kg. Purity, 4 to 8 C.U. cortin/mg. solid.

* All distillations *in vacuo*.

of Na factor. For this reason a method (6) employing this as the initial solvent was used in the early steps of the preparation. The distribution of Na factor and cortin at different stages is shown in Flow Sheet 2, the assays representing averages of 3 to 9 determinations. It will be noted that the first petroleum ether extraction carries with it a considerable amount of Na factor while the second petroleum ether extraction does not. This may be due to the presence of substances which increase the solubility of Na factor in the former. However, back extraction with 10% acetone prevents this loss. The potency of the final product may be 3 to 4 C.U. of cortin and 1.9 U of Na factor per mg.

FLOW SHEET 2

DISTRIBUTION OF THE TWO FACTORS IN THE DIFFERENT STEPS IN THE PREPARATION OF ADRENAL EXTRACT


12 kg. tissue extracted with 24 l. of acetone.
First acetone extract.
Tissue residue extracted with 8 l. of 80% acetone.
Second acetone extract.
Combined extracts reduced to 1/10 volume. Concentrate extracted twice with ¼ vol. petroleum ether.
Residue discarded; cortin, 0/kg.; Na factor, 5.3 u/kg.
Petroleum ether solution.
(Cortin, 3.3 c.u./kg.)
(Na factor, 20 u/kg.)
Petroleum ether solution extracted with ½ vol. 10% acetone.
Acetone solution.
(Na factor 20 u/kg.)
Combined aqueous solutions extracted 4 times with ½ vol. ethylene dichloride.
Residue discarded; both factors absent.
Ethylene dichloride chilled to freeze out the water.
Aqueous sludge discarded.
Ethylene dichloride solution evaporated to dryness* and then extracted with absolute alcohol.
Water discarded.
Water added to make 70% alcohol then extracted with petroleum ether.
Residue discarded.
Water added so that when alcohol is reduced to 10% the extract will represent 60 gm. tissue per cc.
Petroleum ether solution discarded; both factors absent.
Filtrate. Yield: cortin, 66 c.u./kg.; Na factor, 38 u/kg.
Precipitate discarded; both factors absent.

* Increase in purity gained at expense of yield per kg. of tissue.

The final extract obtained by the method outlined in Flow Sheet 2 is subjected to vacuum distillation to remove most of the remaining alcohol. Water is added to the residue to bring it to the original volume. This causes precipitation of about three-fourths of the Na factor together with less than one-tenth of the cortin according to our assays. The precipitate is removed and redissolved in 10% alcohol. This solution can be used for intravenous injection. If administration is to be subcutaneous, further purification must be employed, otherwise a local reaction may appear, which leads to sloughing upon repetition. One of the following methods is used for this purpose, all starting from a 10% alcohol solution.

Ether method. The solution is extracted 6 to 8 times with equal volumes of ether. The Na factor passes into the ether as the alcohol is removed from the aqueous solution leaving some impurities behind. The yield by this method is poor.

Ethylene dichloride method. The solution is extracted 5 times with equal volumes of ethylene dichloride. The water dissolved in the ethylene dichloride is removed by freezing. Then the ethylene dichloride is removed by distillation and the residue dis-

solved in 10% ethyl alcohol. The product is purer than that made by the ether method and the yield is greater.

Charcoal adsorption. The material is adsorbed from solution on activated charcoal (1 gm. for 10 cc.) and eluted with chloroform. Both Na factor and cortin are adsorbed and eluted in the process. Therefore, this will not bring about a separation of the 2 factors. The yield is good but the increase in purity is not remarkable.

Petroleum ether method. The dry ethylene dichloride solution prepared according to the method above is concentrated to small volume. To this is added 9 volumes of petroleum ether (B.P. 30° to 60° C.). The Na factor is precipitated.

A combination of the charcoal adsorption method and the petroleum ether method did not appear to yield a higher degree of purity than the petroleum ether method alone. Na factor extract or precipitate was free from epinephrin according to the Folin, Cannon and Denis method. It was essentially free from cortin according to the cat test.

A comparison of the yields and purity is shown in table 2. The percentage yield is based on the assay of whole extract from which the separation starts.

TABLE 2. Na FACTOR PURIFICATION BY DIFFERENT METHODS (starting with a 10% alcohol solution)

Method	Yield	Purity	
		Start	Finish
	%	u/mg.	u/mg.
Ether	35	0.16	2.6
Ethylene dichloride	71	0.33	7.0
Charcoal adsorption	64	0.53	6.5
Petroleum ether ppt.	80		6.6
	81		6.3
			7.0
			6.6

A study has been made of the distribution of cortin and Na factor in different parts of the adrenal by extracting cortex and medulla separately. These extracts have been compared with extracts of whole adrenal, kidney and brain. The acetone method of extraction was used in all instances, the results being shown in table 3. Kidney and brain extracts were essentially negative. It will be noted that there is no correlation between the cortin and Na factor content of different lots of extract from the same tissue. The medulla contained 30% more Na factor than did the cortex. There are two possible explanations. In the attempt to remove all of the medulla, the inner portion of the cortex is carried along and this may be rich in Na factor, or the medulla may contain Na factor. Because the medulla is never free from cortex it is impossible to determine how much Na factor may be present in the medulla alone. However it seems quite certain from the figures that Na factor is present in the cortex.

There are a number of known substances which will cause retention of Na, some of them having been obtained from the adrenal. We have assayed two of the most potent of the latter.

Seven assays of corticosterone gave an average retention per mg. equivalent to 2.4 u of Na factor. A like number of assays of desoxycorticosterone acetate gave an average retention per mg. equivalent to 7.6 u of Na factor. The latter is of the same magnitude as that obtained for Na factor prepared by one of the better methods (table 2).

TABLE 3. CORTIN AND Na FACTOR CONTENT OF BEEF TISSUES

Adrenal						Kidney	Brain
Whole		Cortex		Medulla			
Cortin	Na factor	Cortin	Na factor	Cortin	Na factor	Na factor	Na factor
c.u./kg.	u/kg.	c.u./kg.	u/kg.	c.u./kg.	u/kg.	u/kg.	u/kg.
75	34	99	50	8	43	3.2	2.0
64	39	66	20	8	35		
64	44	75	60	8	67		
64	33	75	42	5	77		
		96	58	2.6	83		
				2.0	55		
Average							
66.7	37.5	82	46	5.6	60.8		

DISCUSSION

The term cortin was first applied by our group (7) to the hormone in the adrenal cortex necessary for life. Now that it has been shown that there are two factors with functions formerly attributed to one, we have proposed tentatively to limit the term cortin to the factor not concerned with Na metabolism. This factor, as we shall show elsewhere, enables an adrenalectomized animal to maintain health for an indefinite period with low plasma Na. Moreover it is also shown in another report (8) that if an adrenalectomized animal is maintained on an extract to which it has become refractory the plasma Na falls to a level characteristic of adrenal insufficiency without loss of appetite or health.

The term Na factor is convenient because it indicates the essential function involved. This might be applied to a number of substances in the adrenal such as corticosterone and desoxycorticosterone but we believe that it should be reserved for the substance from the adrenal which is most active in Na metabolism, presumably the substance responsible for the development of the refractory state.

The method which we have employed in the assay of Na factor differs somewhat from that of Harrop and Thorn (9). The shorter period of time, 6 hours instead of 24 hours, requires less material and saves time. We have found no greater accuracy with the longer period.

The method described in this paper for the preparation of cortin was designed to remove the Na factor and thus demonstrate the existence of the two hormones. It is not claimed, however, that the extract containing cortin does not contain other substances. It appears to be established from our evidence, that there is a factor in adrenal extract which sustains life of adrenalectomized animals but bears no relation to Na metabolism.

The potency of desoxycorticosterone in retaining Na would lead one to suspect that it was the Na factor. Thorn, Engel and Eisenberg (10) obtained Na retention values for this compound similar to our own. However it must not be forgotten that our purest Na factor contained some impurities. Therefore it would seem that desoxycorticosterone should show relatively greater potency per mg. than it does if it is the Na factor.

It has been impossible to demonstrate that the known crystalline compounds, corticosterone or desoxycorticosterone, bear any relation to the refractory state. This would also indicate that neither one was the Na factor.

SUMMARY

Methods are described for the separation of adrenal extract into 2 fractions, one which maintains the health of adrenalectomized animals but is without effect on Na retention, and the other which is responsible for Na retention. It is suggested that the term cortin be limited to the active principle in the first fraction while the term Na factor be applied to the active principle in the second fraction which is responsible for the development of the refractory state. The potency of the Na factor preparation is of the same magnitude as desoxycorticosterone. Methods for assay of the 2 factors are described. The Na factor is present in both medulla and cortex.

We wish to thank Dr. E. C. Kendall for the corticosterone and Dr. T. Reichstein for the desoxycorticosterone acetate used in this study. We also are indebted to Dr. David Klein of the Wilson Laboratories and Dr. George Cartland of the Upjohn Company for extracts used in part of this research. We likewise wish to express our appreciation for the assays made by Miss Lena A. Lewis and the late Miss Jane E. Gabriel of our laboratory.

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FURTHER STUDIES ON THE REFRACTORY STATE DEVELOPED FOLLOWING REPEATED INJECTIONS OF ADRENAL EXTRACT[1]

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IT HAS BEEN established that large amounts of adrenal extract will cause retention of sodium and increased excretion of potassium when injected into the normal animal (1, 2). However, repeated intravenous or intraperitoneal injections of such an extract become less and less effective until the effect disappears entirely (3). This refractory state does not develop from subcutaneous injections (4). Refractoriness can be conferred on a normal untreated animal by the injection of serum from an animal which is refractory (5). It is species specific. Thus, refractoriness developed against beef adrenal extract fails to confer refractoriness to sheep or horse adrenal extract. An attempt was made to develop refractoriness in a young pig, to pig and to beef adrenal extract, but both were unsuccessful possibly due to the youth of the animal (4).

Studies on refractoriness have been continued and are reported in this paper.

A. Investigation has shown that the pseudoglobulin fraction of the serum from the refractory animal is capable of producing refractoriness in normal animals.

B. A further attempt has been made to develop refractoriness by extract of the same species without success. A sheep was injected with extract of sheep adrenals without development of refractoriness, although refractoriness to beef adrenal extract was produced, by injections of beef adrenal extract.

C. It is also shown in the present report that refractoriness can be developed in animals without adrenals or in patients with Addison's disease.

D. It has been impossible to demonstrate any relation between corticosterone or desoxycorticosterone acetate and the refractory state.

A. Responsibility of the Pseudoglobulin Fraction for Refractoriness

Methods. Serum from 2 dogs known to be refractory to beef and to pig adrenal extract was fractionated into pseudo and euglobulin fractions according to the method used in the preparation of antitoxins (6). Material from 20 to 25 cc. of serum was injected intravenously 10 to 15 minutes before a standard subcutaneous injection of adrenal extract. The electrolyte excretion was determined and compared with that following subcutaneous extract alone or subcutaneous extract and 10 cc. of normal saline intravenously. Methods of analyses are described elsewhere (4).

Results. The results are shown in table 1. The pseudoglobulin fraction of the serum is responsible for the refractory state. If it is injected into a test animal just preceding the injection of an extract to which the donor is refractory, there is little or no Na retention. The euglobulin fraction on the other hand is without effect (table 1, animals *C* and *D*, also table 2, animal 42).

[1] Aided by a grant from the Rockefeller Foundation.
[2] Deceased.

TABLE 1. EFFECT OF SERUM FRACTIONS FROM REFRACTORY DOG ON Na RETENTION IN RESPONSE TO ADRENAL EXTRACT[1]

Serum fraction	Test animal	Na retention, %
None	A	38
Pseudoglobulin	A	1
None	B	39
Pseudoglobulin	B	3
None	C	43
Euglobulin	C	44
None	D	36
Euglobulin	D	36

[1] The same dosage of beef adrenal extract was injected in each test.

B. Inability to Produce Refractoriness by Extract from the Same Species

Methods. One normal adult female sheep was used. Many attempts were made to obtain satisfactory electrolyte balance controls without success. These could be explained by the irregular eating and thus the variable amount of electrolytes absorbed. We finally used the method of passive transfer of the refractory state. In order to reduce the amount of foreign protein as much as possible, the serum was fractionated into the pseudoglobulin and euglobulin fractions by the method already described. Amounts equivalent to 20 cc. of sheep serum were injected intravenously into the dogs 15 minutes before a subcutaneous injection of adrenal extract (10 cc. of sheep adrenal extract or 5 cc. when beef adrenal extract was used). In the majority of cases no ill effects were noted but if the dog showed any uneasiness the test for that day was discarded. After a control period showed that the animal was again in balance, the dog was used for testing. Serum or pseudoglobulin from the sheep was tested (*a*), before she received any injections ("untreated"), (*b*) after 12 intravenous injections of sheep adrenal extract ("sheep extract treated"), (*c*) after 10 intravenous injections of beef adrenal extract ("beef extract treated").

TABLE 2. EFFECT OF SHEEP SERUM FRACTIONS ON Na RETENTION FROM ADRENAL EXTRACTS

Sheep serum fraction	Adrenal extract	Dog tested	Na retention, %
None	Sheep	32	48
Pseudoglobulin untreated	Sheep	32	48
None	Sheep	33	42
Pseudoglobulin sheep extract treated	Sheep	33	44
None	Sheep	16	52
Pseudoglobulin sheep extract treated	Sheep	16	48
None	Beef	37	47
Pseudoglobulin beef extract treated	Beef	37	4
None	Beef	5	51
Pseudoglobulin beef extract treated	Beef	5	12.5
None	Beef	41	36
Pseudoglobulin beef extract treated	Beef	41	7.5
None	Beef	42	36
Euglobulin beef extract treated	Beef	42	48

The sheep adrenal extract was prepared by the method of Cartland and Kuizenga (7) (60 gm. to 1 cc.). The beef adrenal extract was furnished by the Wilson Laboratories (75 gm. to 1 cc.). The intravenous injections were made twice weekly in 10 cc. doses. In earlier work it was shown that the refractory state was developed readily in dogs with either type of extract (4).

Results. The results with the sheep serum are shown in table 2. The pseudoglobulin fraction obtained after repeated intravenous injection of sheep adrenal extract was without effect on the Na retention resulting from sheep adrenal extract. However the pseudoglobulin fraction obtained after repeated intravenous injection of beef adrenal extract greatly reduced or almost obliterated the Na retention from beef adrenal extract. The euglobulin fraction of the same preparation was without effect.

C. *Refractoriness in Adrenal Insufficiency*

All of the earlier studies on the refractory state were carried out on normal

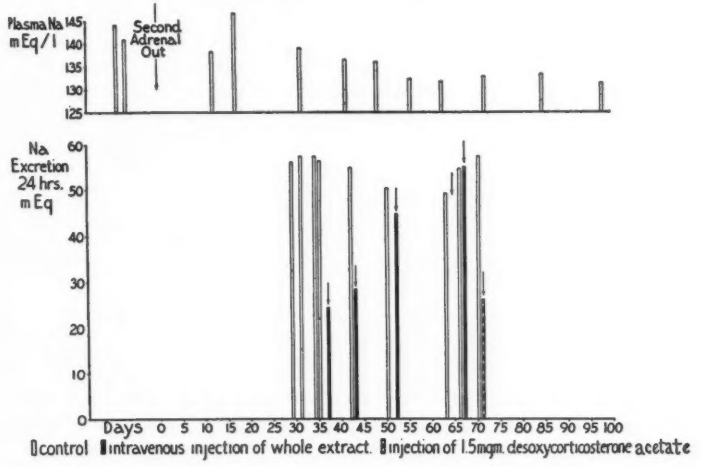

Fig. 1. Reduction in plasma Na in adrenalectomized dog carried on extract to which it is being made refractory.

subjects. They have now been extended to adrenalectomized animals and patients with Addison's disease.

Methods. Control blood studies were made before adrenalectomy. Plasma Na, K, total base; blood urea, red cell percentage, red and white blood cell counts and differential blood cell counts were determined (for analytical methods see an earlier paper, 4). The dogs were adrenalectomized in a 2-stage operation. After complete recovery from the operation and when well maintained on adrenal extract, blood studies were made again. Urine electrolyte excretion was also determined under balanced conditions. The effect of injections of adrenal extract was determined. The 24-hour period recommended by Harrop and Thorn (8) was used for 2 dogs and the 6-hour period previously employed in this laboratory was used for a third dog. Two intravenous injections were made at 12-hour periods on the test days when the 24-hour period was used, while one intravenous injection was made when the 6-hour period was used.

The blood pressures were determined by the method of Parkins (9) using the Dameshek and Loman instrument (10).

Results. Refractoriness to the Na factor is produced in adrenalectomized animals just as easily as in normal animals. This is illustrated in figure 1 from *dog 8* and table 3 from *dog 25*. The results on the third dog were similar. It required intravenous injections on 5 different days, a few days apart to produce complete refractoriness. In figure 1 these injections are indicated by arrows. The same injections served to test the power of Na retention as well as to develop refractoriness. In table 3 the test and injections on the 67th day are not shown. Both chart and table show the fall in plasma Na which accompanies refractoriness provided extract of the same species is used for maintenance. The plasma Na reaches the level found in adrenal insufficiency. It should be noted that when the dog went into heat the electrolytes returned to normal levels, the Na rising and the K falling.

During the whole period of the experiment the dogs remained in good condition. There was no significant variation in weight. The blood pressures showed only slight variations from the normal controls (table 3).

TABLE 3. PLASMA ELECTROLYTES IN AN ADRENALECTOMIZED *Dog 25* BEING TREATED WITH BEEF ADRENAL EXTRACT TO WHICH IT IS MADE REFRACTORY

Days after adrenalectomy	Plasma Na	Plasma K	Blood urea N	Erythrocytes	Na retention following intrav. inj. 5 cc. beef adrenal ext. at 8 A.M. and 8 P.M.	Blood pressure
	m.Eq./l.	*m.Eq./l.*	*mg./100 cc.*	%	%	*mm. Hg*
Before	143–148	4.0–5.0	10–18	43–44		135
52	139	6.4	30	45	42 (54th day)	
59	139	7.1	44	50	23 (60th day)	125 (61st day)
66	144	5.9	28	49	11 (63rd day)	
73	137	6.3	52	42	5 (74th day)	
87	130	6.7	36	43	0 (81st day)	132 (89th day)
115	130	6.9	48	29	51 with 1.5 mg. desoxycorticosterone acetate on 88th day.	
129	142	4.8	32	30		130 (135th day) Dog in heat

With the development of refractoriness there was a definite rise in the blood urea nitrogen. The values from *dog 25* are typical. From a level of 30 mg. per 100 cc. of blood the urea N rose to as high as 58 mg. At no time was there any clinical indication of insufficiency except for a few days when the appetite of *dog 25* was poor. An increase of 50% in the dosage of the extract corrected this. The urea, however, never returned to the normal postoperative level until the development of estrus and pseudopregnancy, when it fell to the pre-refractory range. The plasma Na and blood urea levels showed deviations from normal at essentially the same time during the development of the refractory state. The higher blood urea was not accompanied by a decreased blood pressure.

The blood picture on the development of refractoriness in the adrenalectomized dog showed some tendency for an increase in the percentage of lymphocytes and a corresponding decrease in the neutrophiles. In *dog 8* following adrenalectomy 35 to 39% of lymphocytes were found. After the development of refractoriness the percentage was increased to a range of 60 to 70%. The actual leucocyte count remained essentially constant ranging between 8 and 11 thousand before and after the development of refractoriness.

Addison's disease. Methods. Blood was collected from 2 patients with Addison's disease prior to any intravenous adrenal extract therapy and again after 4 intravenous injections of 10 cc. of adrenal extract administered about 4 days apart. The

sera were separated and tested for their ability to prevent the Na retention effects of a standard dose of adrenal extract. The serum was injected intravenously into cats one-half hour before a standard injection of adrenal extract.

Cats instead of dogs were used for the test. These animals were on a constant diet for several days before use. Normal adult females were fed a constant diet of 100 gm. of beef heart and 50 gm. of salmon 9 hours before the beginning of the test period. Only those animals which consumed their food promptly were considered satisfactory. Fifty cubic centimeters of water was given by stomach tube at the

TABLE 4. EFFECT OF SERA FROM PATIENTS WITH ADDISON'S DISEASE, ON SODIUM RETENTION IN NORMAL CATS INJECTED WITH BEEF ADRENAL EXTRACT

Cat	Serum injection	Na excretion, m.Eq. in 7 hr.	Retention, %
A	10 cc. normal human +extract[1]	controls 3.08–3.50 2.23	32
B	9 cc. Addison A +extract[1]	controls 4.30–4.75 2.62	42
C	12 cc. Addison A after 4 intravenous injections of beef adrenal extract+extract[1]	controls 2.05–2.35 2.10	0
D	10 cc. Addison B +extract[1]	controls 2.38–2.95 1.45	43
E	12 cc. Addison B after 4 intravenous injections of beef adrenal extract+extract[1]	controls 3.38–3.47 2.80	18

[1] 2 cc. of same lot of beef extract. Different cats used each time that extract was injected.

beginning of the test period. The cats were catheterized at the beginning and at the end of the 7-hour experimental period and kept in metabolism cages during that time. The Na excretion for this period was determined by urinary analysis.

Different cats were used for each set of tests because in these animals it seemed best to inject extract intravenously. Subcutaneous injections did not appear to give such uniform results. The single injection method avoided development of refractoriness. Serum was also collected from a normal human subject in order to determine any possible effect of normal human serum on cat electrolyte excretion or response to extract.

Results. The injection of normal human serum alone was without effect on the excretion of Na. A cat excreted 3.40 and 3.12 m.Eq. during a 7-hour period on different days. On the day that 10 cc. of normal human serum was injected the Na excretion for a similar period was 3.33 m.Eq. Likewise normal human serum appeared to have no effect on the ability of adrenal extract to cause retention (table 4, *cat A*).

Injection of sera obtained from patients before they were treated intravenously did not seem to modify the retention effect of extract (*cat B, D*). However sera from the same patients after intravenous extract treatment did reduce the effect of adrenal extract on retention (Addison *A*, *cat C* and Addison *B*, *cat E*).

D. Absence of Relationship Between Corticosterones and Refractoriness

The active crystalline compounds which have been isolated from the adrenal appear to have no relation to the refractory state. After refractoriness has developed the response to desoxycorticosterone acetate is not reduced. Two dogs made completely refractory to beef adrenal extract gave excellent retention effects from 1.5 mg. of this crystalline substance (fig. 1, table 3).

The refractory state failed to develop in a normal female dog following 4 intravenous injections of 4 mg. of corticosterone in 10% alcohol. Four injections of standard adrenal extract were sufficient to confer complete refractoriness. A Na retention of 29% was obtained on the initial injection and 27% on the final injection of corticosterone.

DISCUSSION

Our observations indicate that the refractoriness from adrenal extract is an immune reaction. The responsibility of the pseudoglobulin fraction for the passive transfer of the refractory state is significant. Likewise the inability to develop refractoriness by extract from the same species conforms to a reaction of this type. The close relationship between the refractory state developed to the Na factor and other immune reactions is in agreement with the interpretation of the antihormone problem presented by Gordon et al. (11). They have shown that the reticulo-endothelial system participates in the production of gonadotropic and thyrotropic antihormones as in antibody formation.

The recent report by Gordon et al. (12) that the antigonadotropic principle is more readily elaborated in the mature than in the immature rat is in agreement with our earlier observation (4) that refractoriness failed to develop in the immature female pig, repeatedly injected intravenously with beef adrenal extract. This property of the mature animal to elaborate the inhibitory factor more readily than the immature is also true in many standard immune reactions.

It is uncertain whether desoxycorticosterone is the active Na retaining part of the Na factor. If so, it is possible that a haptene combination exists between desoxycorticosterone and a protein and that the combination is broken in the process of isolating the desoxycorticosterone. The ability to confer refractoriness always seems to exist in an adrenal extract which is potent for Na retention. The failure to develop refractoriness to corticosterone but to develop it to extracts prepared by less rigorous treatment is similar to the findings in antithyrotropic production. Werner (13) observed that the more highly purified thyrotropic hormone preparations were ineffective or much less effective in the production of antihormone than the less purified preparations. In this case, as in the case of the Na factor it would appear that some protein material, specific for the species from which the glands were obtained, was broken off from the active part of the molecule in the process of purification. It is quite possible that the Na factor is unrelated to the known compounds.

The ability to develop refractoriness in the adrenalectomized animal by repeated intravenous injections of adrenal extract is of particular interest in view of the fact that the plasma electrolyte picture in animals thus made refractory is similar to that of the non-refractory adrenalectomized animal permitted to develop insufficiency. The animal is maintained in good condition in spite of the low plasma Na if the dosage used in treatment is increased. This would indicate two essential factors, one which controls Na metabolism (Na factor) capable of producing a refractory state and the other (cortin) which supports life but has nothing to do with the electrolytes and apparently does not develop the refractory state. The increased require-

ment of extract after the development of refractoriness when the Na factor becomes ineffective indicates synergism between the two factors.

It is interesting that the refractory state in no way interferred with the maintenance of the adrenalectomized pseudopregnant dog without extract. The normal plasma electrolyte picture shows that the refractoriness was not effective against the female sex hormones which maintain the adrenalectomized dog during the period of pseudopregnancy.

The development of refractoriness by intravenous injection in Addison's disease makes it inadvisable to resort to this method of injection except in severe crisis. If this method is necessary, extract from a species usually not employed in routine treatment should be used. Either pig or sheep adrenal extract is advisable because the ordinary commercial extract is made from beef adrenals.

SUMMARY

The pseudoglobulin fraction of the blood serum from animals made refractory to the Na factor, is responsible for the refractory state.

It has been impossible to develop refractoriness by adrenal extract of the same species as the subject.

Refractoriness to the Na factor is produced in adrenalectomized animals just as easily as in normal animals. As refractoriness develops the plasma sodium falls until it reaches the level characteristic of adrenal insufficiency. In spite of this the animal remains in good condition provided the amount of extract is increased.

The increased requirement of extract after the development of refractoriness indicates synergism between the Na factor and cortin.

The refractory state did not interfere with the maintenance of the adrenalectomized pseudopregnant dog without extract. The normal plasma electrolyte picture shows that the refractoriness was not effective against the female sex hormones responsible.

The development of refractoriness by intravenous injection in Addison's disease makes such injection inadvisable unless extract of another species than that used for maintenance is employed.

It has been impossible to show any relationship between the corticosterones and refractoriness.

Our evidence indicates that refractoriness to the Na factor is an immune reaction.

We wish to thank Dr. E. C. Kendall for the corticosterone and Dr. T. Reichstein for the desoxycorticosterone acetate used in this study. We are indebted to Dr. David Klein of the Wilson Laboratories for extracts used in part of this research. Our appreciation is also due Mr. H. J. Spoor of our own laboratory for the making of the sheep adrenal extract. Dr. W. A. Starin has kindly given advice on certain phases of our problem.

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COMPENSATORY HYPERTROPHY OF THE DOG ADRENAL GLAND FOLLOWING UNILATERAL ADRENALECTOMY[1]

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DIAMETRICALLY OPPOSED conclusions have been reached relative to the presence or absence of compensatory hypertrophy in the remaining adrenal gland following unilateral adrenalectomy in the rat, guinea pig and rabbit. Harley (1) and Elliott and Tuckett (2) found no evidence of enlargement of the remaining rat adrenal gland after unilateral adrenalectomy. Wiesel (3) and Scott (4) observed an augmentation of accessory cortical tissue after unilateral adrenalectomy in the rat; these authors accounted for the negative results obtained by the former workers as being due to enlargement of accessory cortical tissue. On the other hand, MacKay (5) found a compensatory hypertrophy in the rat adrenal which amounted to a 61.0% total enlargement and a cortical enlargement of 91.0%. More recently MacKay (6) reported that he was able to suppress the compensatory enlargement by administration of cortical extract. Ingle (7) found that if the diet of unilateral adrenalectomized rats contained adequate sodium, adrenal hypertrophy did not occur. Hypertrophy did occur, however, in similar animals subjected to the stress of work or extremes of temperature.

Stilling (8) observed compensatory enlargement in 6 rabbits and Simmonds (9) obtained the same results in 6 rabbits and 2 guinea pigs. On the other hand, Boycott and Kellaway (10) concluded that there is no compensatory hypertrophy in the rabbit (43 animals). MacKay (5) disagreed with Boycott and Kellaway's interpretation of their data and believed that they did find compensatory enlargement.

It has been suggested (3, 4) that in some instances the conflicting results may be due to enlargement of accessory cortical tissue. Since accessory cortical tissue is much less common in the dog than in other laboratory animals (11, 12), a study of this question in the dog should provide definitive evidence regarding this point. Accurate volumetric data pertaining to this subject are not available.

MATERIALS AND METHODS

Sixteen healthy adult dogs of mixed breed were used. The animals were fed the diet of mixed hospital kitchen scraps that is given to the dog colony. Eight male and 8 female animals were unilaterally adrenalectomized. In 4 animals of each sex the right gland was removed and in 4 the left gland was extirpated. After a postoperative period varying from 3 to 90 days, the animals were killed and the remaining gland was excised at autopsy. No accessory cortical tissue was observed either at the time

[1] The word hypertrophy is used here with an anatomical connotation indicating an increase in the size of the gland by enlargement of its constituents cells without regard to physiological changes which may have occurred.

of the operation or during the autopsy. Since there was no significant difference in the per kg. weights of the right and left gland of the dog (13), the gland removed surgically was used as a normal control for comparison with the gland removed at varying periods postoperatively. The average weight of the dogs was only 0.1 kg. greater at autopsy than at the time the normal gland was removed. Since the animals practically maintained their weight during the postoperative period the size of the adrenals is given in cu. mm. rather than units per kg. of body weight.

The glands were carefully cleaned, weighed to the mg. and the specific gravity obtained by the suspension method (14). The adrenals were then fixed in formalin, dehydrated in dioxan, embedded in paraffin and serially sectioned at 20 micra. Every 10th section was mounted and stained with hematoxylin and eosin. The sections were projected on heavy paper at 10 diameters enlargement and the outlines of the cortex, cortical zones and medulla were drawn. The area of the section drawings was measured with a planimeter. In order to compensate for shrinkage which occurred the computed volumes were calculated to correspond with the true volumes as obtained from the weight and specific gravity.

RESULTS AND DISCUSSION

Although Baker (13) found no statistical difference in the per kg. weight of the right and left adrenal glands of the dog (1006 animals), he did find the right gland larger than the left one in 56.0% of the males and in 54.0% of the females. Of the 16 glands removed surgically, the average volume of the right glands was 10.5% larger than the left ones. The medulla constituted 11.8% of the total gland on the right side and 15.0% on the left side (fig. 1, table 1). In the right glands the average total amount of cortex was 13.7% greater than in the left glands. In the 16 normal glands removed surgically the zona glomerulosa averaged 23.3% (112.3 cu. mm.), the zona fasciculata 68.9% (339.1 cu. mm.) and the zona reticularis 7.5% (37.6 cu. mm.) of the total volume of cortex (493.1 cu. mm.).

In every animal except one, the gland removed at autopsy was larger than the normal gland removed surgically, The hypertrophy ranged from 8.8% to 22.8% and averaged 13.4%. In one animal the gland removed at autopsy was 2.7% smaller than the opposite gland removed surgically. Histological study of this gland and measurements of the cortical cells indicated that hypertrophy had taken place. The enlargement which occurred, however, was apparently insufficient to overcome an unusual size disparity which existed between the right and left glands.

The normal male glands averaged 619.1 cu. mm. whereas the glands removed at autopsy averaged 726.5 cu. mm. representing an increase of 14.7%. The cortex enlarged by 17.1% and the medulla decreased by 3.7%. The normal female glands surgically removed averaged 521.0 cu. mm. whereas those removed at autopsy averaged 590.0 cu. mm. representing an 11.7% increase. The cortical portion of the female glands enlarged by 13.7% while the medulla decreased by 4.0%.

Combining the figures for the two sexes, the average volume of the cortex of the glands removed at autopsy was 13.4% greater than that of the normal glands while the volume of the medulla averaged 3.7% less than the normal glands (table 1, fig. 1). With respect to the layers of the cortex, it was found that the average volume of the zona glomerulosa was 20.2% greater and the zona fasciculata 17.1% greater in the enlarged glands than in the normal glands. The average volume of the zona reticularis was 20.7% less in the enlarged glands than in the normal ones. The medullary tissue appeared histologically to be slightly more compact in the hypertrophied glands. The glandular enlargement was probably not affected by any weight change in the ani-

mals for the original weights were practically maintained throughout the experimental period.

Careful measurements of the size of the cells in the zona glomerulosa and zona fasciculata indicate that the enlargement was due to an increase in the size of the cells in these two layers of the cortex. No increase in the number of mitotic figures could be observed in the hypertrophied glands.

These data suggest that there is a moderate compensatory enlargement in the

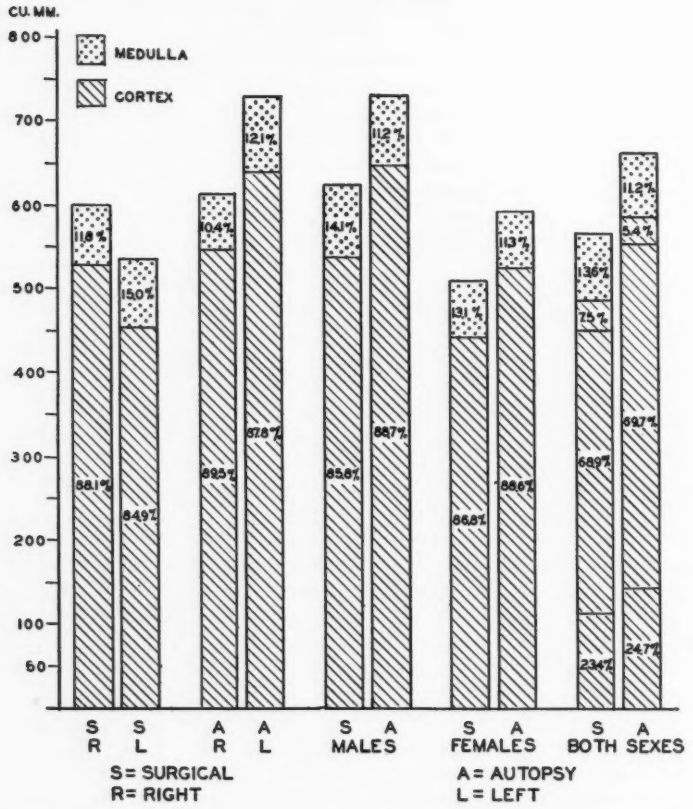

Fig. 1. AVERAGE VOLUMETRIC COMPARISON OF THE RIGHT AND LEFT NORMAL ADRENAL GLANDS WITH RIGHT AND LEFT HYPERTROPHIED GLANDS AND NORMAL MALE AND FEMALE GLANDS WITH ENLARGED MALE AND FEMALE GLANDS. The figures within the columns combining the sexes represent percentages of the cortical zones to the total cortex (zona glomerulosa at the base of column) in the remaining columns they indicate percentage of cortex and medulla of total gland volume.

dog adrenal gland which apparently involves only the zona glomerulosa and zona fasciculata of the cortex. The decrease in volume of the zona reticularis might be accounted for by assuming that the increased volume of the outer two layers of the cortex encroaches centrally to some extent. However, such a process would tend to be self limiting due to the decreasing blood supply. These results in the dog contrast sharply with the negative data of Harley (1) and Elliott and Tuckett (2) who found

no hypertrophy in the rat and MacKay (5) who found a 91.0% cortical hypertrophy in the rat.

The augmentation of the remaining gland apparently begins about 7 or 8 days after unilateral adrenalectomy. The glands of one dog autopsied 3 days and another 5 days after unilateral adrenalectomy showed no compensatory hypertrophy (glands not included in table or figure). Another gland removed 7 days postoperatively showed a 15.6% enlargement and in two other animals the glands had enlarged

TABLE 1. VOLUMETRIC COMPARISON OF THE TOTAL VOLUME, CORTEX, CORTICAL ZONES AND MEDULLA OF THE NORMAL AND HYPERTROPHIED DOG ADRENAL GLANDS. ALL FIGURES REPRESENT AVERAGES OF THE GROUPS INDICATED

	Total volume	Medulla		Cortex		Cortical zones			Cortico-medullary ratio
		Volume	% whole gland	Volume	% whole gland	Z. glomerulosa	Z. fasciculata	Z. reticularis	
	cu. mm.	cu. mm.		cu. mm.		cu. mm.	cu. mm.	cu. mm.	
				Normal glands					
R. glands	596.6	70.9	11.8	525.6	88.1	113.8	370.7	40.6	7.4:1
L. glands	533.6	80.1	15.0	453.5	84.9	110.9	307.5	34.6	5.6:1
% difference	10.5 R>L	11.4 R<L		13.7 R>L		2.5 R>L	17.0 R>L	14.7 R>L	24.3 R>L
Average	565.1	75.5	13.4	489.5	86.5	112.3	339.1	37.6	6.5:1
				Male glands					
Surgical	619.1	84.9	13.7	534.1	86.2	122.0	368.2	43.5	6.2:1
Autopsy	726.5	81.7	11.2	644.7	88.7	157.8	455.5	31.0	7.8:1
% difference	14.7 A>S	3.7 A<S		17.1 A>S		22.6 A>S	19.1 A>S	28.7 A<S	20.5 A>S
				Female glands					
Surgical	521.0	69.0	13.2	452.0	86.7	107.7	309.3	34.7	6.5:1
Autopsy	590.0	66.2	11.2	523.7	88.6	130.0	362.3	31.1	7.9:1
% difference	11.7 A>S	4.0 A<S		13.7 A>S		17.1 A>S	14.6 A>S	10.3 A<S	15.8 A>S
				Both sexes					
Surgical	570.0	76.9	13.4	493.1	86.5	114.8	338.7	39.1	6.4:1
Autopsy	658.2	74.0	11.2	584.2	88.7	143.9	408.9	31.0	7.8:1
% difference	13.4 A>S	3.7 A<S		15.6 A>S		20.2 A>S	17.1 A>S	20.7 A<S	18.6 A>S

A=Hypertrophied glands removed at autopsy
S=Normal glands removed surgically
R=Right glands
L=Left glands

11.2% after a 90-day postoperative period. The appearance of the hypertrophy seems to coincide in time with the deficiency symptoms produced by bilateral adrenalectomy. It must be pointed out that the data are necessarily static and therefore do not preclude the possibility of increased secretion rates or a changed functional metabolic level.

SUMMARY

After unilateral adrenalectomy of the dog, the remaining gland underwent a moderate amount of enlargement, averaging 15.6%. The enlargement was found in the zona glomerulosa and zona fasciculata of the cortex.

The hypertrophy which occurred in the two outer cortical zones was apparently at the expense of the zona reticularis. The medulla appeared to be slightly more compact in the enlarged glands.

There was no essential difference in the hypertrophy of the adrenals in the two sexes.

The hypertrophy occurred rapidly about the time signs of cortical deficiency would occur in the totally adrenalectomized animal and did not appear to change significantly during long postoperative periods.

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THE REACTION TO PITUITRIN AND ADRENALIN OF THE MYOMETRIUM OF THE DOMESTIC SOW

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In 1927, KNAUS (1) announced his discovery of the remarkable change which takes place in the rabbit's uterus at the advent of pregnancy, rendering the myometrium insensitive to doses of pituitrin sufficient to call forth large contractions in the non-pregnant state. By the time the Knaus effect was conclusively demonstrated as a function of the hormone of the corpus luteum, species other than the rabbit had been investigated. All of these species examined fell into two general types, so far as reactivity to pituitrin during pregnancy was concerned. In one group, exemplified by the rabbit, the hormone of the corpus luteum inhibited the uterine contractions normally evoked by small doses of pituitrin. The characteristic response of the second group was first found in the rat (2), and later in the guinea pig and mouse. In this group the uteri respond to pituitrin at all stages of the sexual cycle and during pregnancy.

TABLE 1

	PITUITRIN			ADRENALIN	
	Non-gravid	Gravid		Non-gravid	Gravid
Rabbit, cow cat, squirrel, man	Motor	Non-motor	Rabbit, squirrel, hedge hog, monkey, man	Motor	Motor
Guinea pig, rat, mouse	Motor	Motor	Guinea pig, rat, mouse	Inhibitor	Inhibitor
			Cat, dog	Inhibitor	Motor

The effect of adrenalin upon the uterine muscle of different species is more varied. According to the simplest schema (3) there are three types. In the first group, typified by the rabbit (1), the uterine muscle responds to adrenalin with strong contractions, both in the pregnant and non-pregnant states. The second group includes the guinea pig, rat and mouse, the uteri of which respond to adrenalin with marked loss of tonus and greater quiescence both in pregnant and non-pregnant animals (4). In the third group, made up of the cat (5) and the dog, adrenalin acts on the non-pregnant uterus to produce pronounced relaxation, and on the pregnant uterus to produce distinct contractions.

Table 1 (from data cited by Reynolds, 3) summarizes the effects of adrenalin and pituitrin on the gravid and non-gravid uteri of various species.

It seemed of interest to investigate these relations in an animal such as the domestic sow, whose sexual cycle has been well studied from many points of view; accordingly the response of the sow's uterine muscle to adrenalin and pituitrin was tested throughout the estrous cycle.

Fresh sows' uteri with ovaries attached were collected at a nearby abattoir, and kept under refrigeration until ready for use. In all but a few cases this period did

not exceed 2 hours. A longitudinal strip, of uniform dimensions in each case, was then cut from the antemesometrial border of one cornu, and suspended immediately, in the usual way, in a 250 cc. bath of oxygenated mammalian Locke's solution at 37.5° C. made up as follows: NaCl .90%; KCl .042%; $MgCl_2$ .0005%; $CaCl_2$ .024%; $NaHCO_3$ .050%; Glucose .003%. In all cases the strip of muscle became spontaneously active within a short time.

After sufficient time had elapsed to allow the muscle to record recurring spontaneous contractions, a standard dose of one international unit of pituitrin was added to the bath. When a record had been made of the effects of this, 1 cc. of 1:10,000 adrenalin chloride was placed in the Locke's solution. On occasion, the order of addition of pituitrin and adrenalin was reversed; or the bath was changed between each dose. The results seemed no different from those obtained with the procedure first mentioned.

In this way, records were taken from a total of 31 uteri. Of these, 2 were from pregnant animals, 19 from non-pregnant animals whose ovaries contained large, supposedly functional corpora lutea, and 10 from animals whose ovaries showed large follicles.

Each uterus was assigned its proper date in the sexual cycle. Those from the 16th day on were dated by gross inspection of the follicles, and the pregnant uteri by the size of the embryos. The remaining uteri were dated by Corner's (6) method, involving histological examination of the sectioned corpora lutea and uterus.

The effect of pituitrin. Only a superficial examination of the kymograph records was necessary to reveal that, as between the two patterns of response to pituitrin mentioned above, the domestic sow belongs in the group occupied by the guinea pig, rat and mouse, for even with the relatively small dosage of pituitrin used, there was no single uterus, whether pregnant or not, whether under the influence of actively secreting corpora lutea or actively secreting follicles, that failed to contract, more or less markedly, upon the administration of pituitrin.

Qualitatively, the response to pituitrin was characteristic in almost every case. It took the form of an immediate upward sweep of the recording lever, as the muscle suddenly contracted; followed by additional contractions, usually of heightened frequency, the increased tonus of the muscle gradually diminishing until the lever approached the former base-line.

In analyzing the results the response to pituitrin was graded, according to its magnitude, as +, ++ or +++ (fig. 1, 2, 3). The results for each uterus are tabulated in table 2.

In the summary of table 2 the uteri have been divided into 2 groups: in one, the corpora lutea were active endocrine bodies (3rd to 15th day and pregnancy); in the other they were not; and the percentage of each of the 3 graded responses occurring in each group has been computed. It appears from this that not only is the reactivity to pituitrin of the sow's uterine muscle not inhibited in the presence of active corpora lutea, but on the contrary, it is actually enhanced. About 70% of those specimens dated from days 3 through 15 in the cycle (including the 2 pregnant uteri) showed a +++ response to pituitrin, while only about 30% of those from the other days of the cycle responded with the same intensity (fig. 1, 2, 3).

Although the number of uteri examined is too small to permit decisive conclusions, these results suggest that the sow may represent a third and new group in addition to the two mentioned above, a group in which the presence of progesterone in the body, instead of inhibiting, heightens the sensitivity of the myometrium to pituitrin.

The effect of adrenalin. In all of the uteri studied, regardless of date in the cycle

or condition of pregnancy, the effect of administering adrenalin was qualitatively the same. The reaction was registered as one of diphasic shape: a sharp contraction occurs, followed immediately by a marked loss of tonus, increased quiescence, and generally, a slackened frequency of any spontaneous contractions which might persist.

TABLE 2

Pig no.	Day in cycle	Reaction to pituitrin
13	3–6	++
22	3–6	+++
27	3–6	+++
31	3–6	+++
5	6–8	+++
30	6–8	+++
7	8–10	++
8	8–10	+++
9	8–10	+++
10	8–10	++
12	8–10	+++
4	8–10	++
24	8–10	+++
26	8–10	+++
6	10–15	+++
21	10–15	+++
25	10–15	+++
11	15–16	+
28	15–16	+++
2	17	++
15	17	++
16	17	++
19	18	+++
20	18	+++
14	19	+
1	20	+
17	20	++
18	20	++
23	pregn. 4th wk.	++
29	pregn. 3rd wk.	+++

Summary

	Percentage		
	+	++	+++
Active corpora lutea (3 to 15 day and pregn.)	0	26.3	73.7
Inactive or degenerating corpora (16 to 20 d.)	27.3	45.4	27.3

TABLE 3

Pig no.	Day in cycle	Reaction to adrenalin
13	3–6	–––
22	3–6	–––
27	3–6	––
31	3–6	–––
5	6–8	–––
30	6–8	––
7	8–10	––
8	8–10	–––
9	8–10	–––
10	8–10	––
12	8–10	––
24	8–10	–––
26	8–10	–––
6	10–15	–––
21	10–15	–––
25	10–15	–––
11	15–16	–––
28	15–16	–––
2	17	–––
15	17	––
16	17	–
19	18	––
20	18	––
3	19	–––
14	19	––
1	20	––
17	20	–
18	20	–––
23	pregn. 4th wk.	–––
29	pregn. 3rd wk.	–––

Summary

	Percentage		
	–	––	–––
Active corpora lutea (3 to 15 day and pregn.)	0	27.8	72.2
Inactive or degenerating corpora (16 to 20 d.)	16.6	41.7	41.7

– Slight relaxation
–– Distinct relaxation
––– Great relaxation

The method of classifying the response of each uterus to adrenalin was the same as that employed for pituitrin, each being graded according to the degree and duration of the relaxation. The results are summarized in table 3 and illustrated in figures 4, 5, 6.

When each uterus is placed into one of two groups, depending on whether or not it was taken from an animal with active corpora lutea, and the percentage of well marked responses to adrenalin (indicated in the table by 3 minus signs) is computed for each of these groups (summary of table 3), it seems probable that the character-

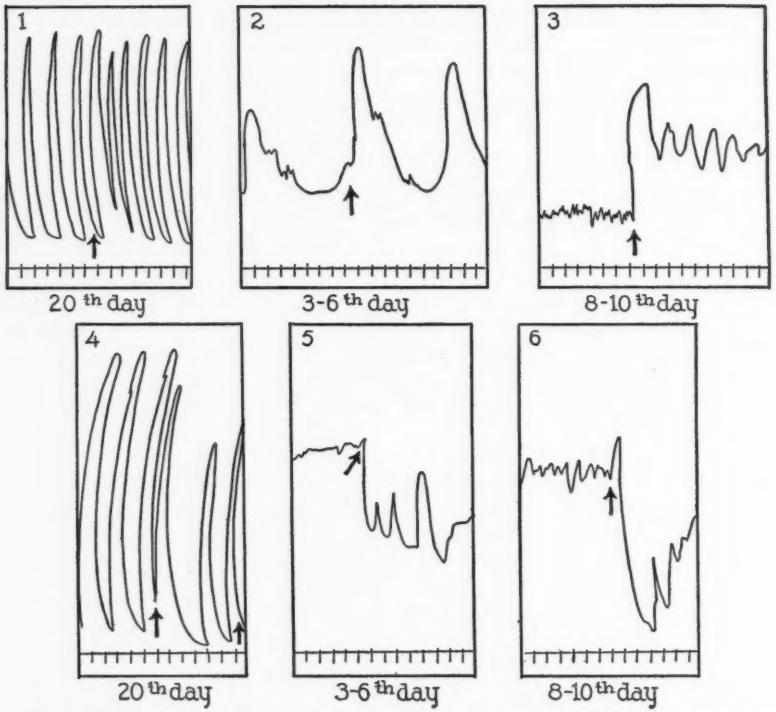

Fig. 1–6. Reproduced from kymograph records. Time is in minutes. Arrow indicates time at which 1 I.U. of pituitrin (fig. 1, 2, 3) or 1 cc. of 1:10,000 adrenalin chloride (fig. 4, 5, 6) was added to bath. Fig. 1, 2, 3 represents a +, ++, +++ reaction respectively. Fig. 4, 5, 6 a −, − −, − − − reaction respectively. Numbers under each figure indicate date in cycle.

istic reaction of the myometrium to adrenalin is heightened under the influence of the corpus luteum.

In general, then, it can be definitely stated that the sow belongs in the same group with the guinea pig, rat and mouse, in which the effect of adrenalin is inhibitory in both the gravid and non-gravid states. The results tabulated above, however, suggest in addition, that unlike the other members of this group, the activity of the uterine muscle of the sow may be more intensely inhibited by adrenalin at those stages in which the corpus luteum is actively secretory.

SUMMARY

The sow's uterine muscle is found to respond to pituitrin with contractions, and to adrenalin with relaxation, at all stages of the cycle. Less definitely, the results suggest that the sow's myometrium shows its characteristic reaction to the two drugs more intensely when the corpora lutea are active than at other times.

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THE CELL CONTENTS OF THE CORTEX OF THE SUPRARENAL GLAND[1]

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AN EXAMINATION of three malignant cortical suprarenal tumors in females (1, 2, 3) all manifesting virilism, showed, with Kull's technic for staining, discrete fuchsinophil granules present in the cells. On the other hand a similar tumor in a male manifesting feminism (4) showed no such granules.

These findings suggested the possibility that sex hormones were associated with these granules and an investigation was carried out on the routine postmortem material to ascertain whether or not the cell content varied with the sex.

Suitable postmortem material was examined from autopsies performed at the Royal Free Hospital during the years 1935 to 1938 inclusive. Preliminary studies of

TABLE 1. ANALYSIS OF CASES IN AGE GROUPS

Age groups	Males			Females			Total males and females		
	Total cases	Granules present	% granules present	Total cases	Granules present	% granules present	Total cases	Granules present	% granules present
0– 1	13	—	0	6	—	0	19	—	0
1–10	2	—	0	5	—	0	7	—	0
10–20	11	—	0	3	—	0	14	—	0
20–30	8	1	12.5	18	4	22	26	5	19
30–40	6	2	33	13	3	23	19	5	26
40–50	13	5	38	7	2	28	20	7	35
50–60	34	10	30	14	6	42	48	16	33
60–70	26	9	34	20	9	45	46	18	39
70–80	4	2	50	2	1	50	6	3	50
80–90	3	—	0	—	—	—	3	—	0

these glands undertaken by Ross (4) gave the impression that differences which were fairly constant existed between the cortical cells of both sexes, no granules being found in females under 50 years of age, while some males showed granules limited to the innermost layer of the cortex after puberty, none being found in the adrenals of still-born or young infants of either sex. As the investigation proceeded further, however, it was found that this was not borne out, as table 1 and figures 2 and 3 show.

Twenty-five per cent of all cases examined showed the presence of granules, women giving a slightly higher percentage than men. No granules were found in the

[1] Grateful acknowledgment is hereby made to Dr. Joan Ross for the material which she put at my disposal and for her help and advice throughout this investigation.
[2] Mabel Webb and A. M. Bird Research Scholar.

adrenals of either sex under the age of 20. In the age groups 20 to 40, 22.2% showed granules; in the 40 to 60 group the percentage was 34; and in the age group 60 and over it was 38. Application of the χ^2 test to these last 3 age groups gives a P. of 0.02.

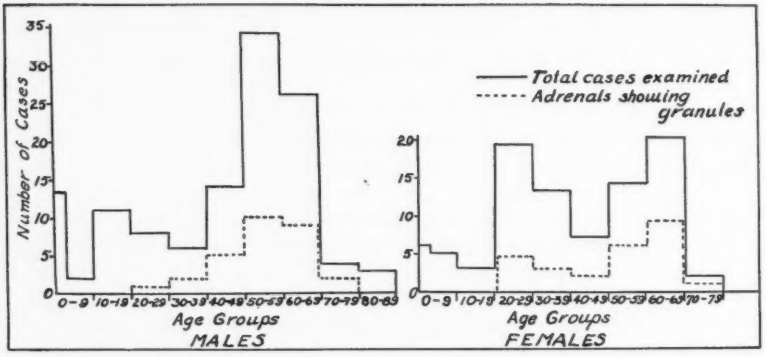

Fig. 1. Curves showing age incidence of necropsies examined and those cases showing granules in the suprarenals.

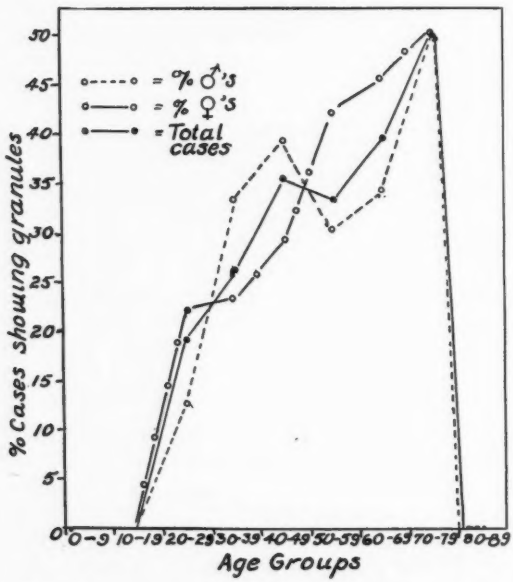

Fig. 2. Curves showing age incidence of percentage of cases showing granules in suprarenals.

Thus the difference observed between the age groups is very unlikely to be the result of random sampling.

The suggestion that granules in males were limited to the innermost layer of the cortex only, was not confirmed. Granules found, whether in males or females, were

usually scattered throughout the cortex. In cases where they were present in abundance, the granules were mostly found in patches extending throughout the depth of the cortex, though on the whole the zona glomerulosa or outermost zone showed the fewest.

No granules being found in the adrenal cortex of stillborn or young infants of either sex, an investigation was attempted on fetal adrenals as far as this material was available; the necessity for the tissue being very fresh rendering the glands obviously difficult to obtain. In all, 6 cases were examined and the glands stained both by Kull's method and the ponceau fuchsin stain employed by Vines (5). Table 2 gives the results which were obtained.

TABLE 2

Sex	Age, wk.	Kull's stain	Ponceau fuchsin stain
—	6	No granules	Negative
Male	12	No granules	Negative
Female	14	No granules, heavy patchy fuchsinophil staining in fetal cortex	Faintly positive
Male	16	Entire fetal cortex a mass of granules	Strongly positive
Male	18	No granules	Negative
Male	20	No granules	Negative

Broster and Vines (5) in their investigations on a small number of fetal adrenals, suggested that a male phase (i.e. when the ponceau fuchsin stain was positive) existed between the ages of 9 and 17 weeks in males and between the 11th week and about the 14th week in females. It will be seen that the results obtained from these few cases correspond with those of Broster and Vines. It seems very difficult to explain, however, why if the presence of granules and a positive ponceau fuchsin stain correspond to the presence of male hormones, they should so consistently be absent at full term in both sexes, indicating that the male phase is transitory in both male and female.

An attempt was made to ascertain whether the granules could be attributed to any other cause. It was suggested that granules were not present in cases where cortical cells showed the presence of lipoid: a careful scrutiny, however, showed that though this was not often the case, a number showed clear discrete granules in the open lipoid cells. The cases were then grouped under the diseases causing death and table 3 compiled.

TABLE 3. ANALYSIS ACCORDING TO DISEASE

Disease	Males		Females		Total male and female
	Total cases	% granules present	Total cases	% granules present	% granules present
Malignant tumors	30	41.0	7	28	39.0
Infective (acute)	20	33.0	18	30	31.0
Infective (chronic)	8	37.5	7	14	27.0
Cardiovascular	24	35.5	16	20	29.0
Trauma	7	0	1	100	12.5
Thyrotoxicosis	1	0	8	50	44.0

Since the quantity of the granules varied considerably in positive cases, a list was drawn up of those glands showing many granules, those with scanty or indefinite ones being ignored. Nineteen cases in all were found with abundant granules, 10

being males and 9 females. Of these, 12 showed severe infective conditions, 4 malignant disease, 2 of which had superimposed infection, one was a case of chloroform poisoning, and the 5 remaining were endocrine disturbances. The high incidence of severely infected cases among those with granules is interesting.

The high incidence of granules among the few thyrotoxic cases examined led to a careful scrutiny for any special histological feature but nothing of note was discovered.

New growths and nodules. Nodules, islets of suprarenal cortical cells for the most part with a definite zonal arrangement and surrounded partly or entirely with fibrous tissue, were found in 64 out of 214 cases. making a percentage of 30, which is considerably lower than the 75% found by Vines (6) in his investigations. An analysis of these nodules into age groups showed them to be fairly evenly distributed throughout life. Granules were present only in those nodules which were found in glands which also showed granules.

After eliminating those glands which macroscopically were cortical adenomata but histologically appeared to be merely hyperplasia of the cortical tissue, 6 cortical adenomata were discovered, one case was bilateral, so that 5 cases out of the 200 showed tumors. The size of the adenomata varied from 4.5 cm. to 2.5 cm. across and were found in patients between the ages of 50 and 66, equally distributed between the two sexes. Only one of these tumors showed the presence of granules, but no evidence of virilism was recorded in the history of the patient.

Other primary tumors found in the series included a small lipoma situated at one pole of the gland with a thin rim of cortical tissue surrounding it and a neuroblastoma in a young child.

Eight of the thirty-six malignant cases examined showed the presence of secondary growths in the suprarenals. In view of the very definite statement made by Willis (7) regarding the rarity of cortical invasion, it was a little surprising to find that in six out of the eight cases, the cortex was undoubtedly involved, in some cases nodules of growth were present in the cortical tissue and in others either the whole gland or one pole was replaced by growth.

Animal investigations. The suprarenals of 20 mice were fixed in Helly's fluid and stained by Kull's method for granules. The difficulty met with in obtaining sections which included the medulla in such small adrenals made the study somewhat unprofitable. In most of the cases in which the medulla was visible, there was a deeply staining zone in the depth of the cortex which appears to correspond to the 'androgenic' zone in mice described by Grollman (8) but in no case were granules found and the investigation was abandoned.

A series of guinea pig adrenals were examined and granules found in all adult glands of either sex and in every case were present in the deep cortical zone. Granules in the male guinea pig suprarenals were more abundant than in the female and some degree of fatty degeneration was noticed in the cortex of every female gland examined whereas it was rare to find this condition in the male.

SUMMARY

Discrete fuchsinophil granules were found in certain malignant cortical suprarenal tumors in females manifesting virilism, which led to an investigation being carried out on routine postmortem suprarenals to ascertain whether these granules were in any way associated with sex hormones.

The granules were found to be present in 24% of adult male suprarenals examined and in 28% of female suprarenals. The ages ranged between 24 and 72 years in

females and 27 and 75 in males. In females the percentage of glands with granules showed a steady increase with age. In males there was a similar but less regular increase with age, the irregularity being probably due to random sampling.

With the exception of the gland of a 16-week-old fetus, which showed granules in abundance, in no case under 24 years were they apparent. It was concluded therefore that age has some influence on the presence of these granules, but sex plays no part.

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THE EFFECT OF ADRENALECTOMY ON THE KETOSIS PRODUCED IN RATS BY ANTERIOR PITUITARY EXTRACT[1]

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IT WAS FIRST SHOWN by Fry (1) that removal of the adrenal cortex brings about a reduction of the ketonuria which occurs in fasting rats after the injection of anterior pituitary extract. Confirmatory reports have subsequently appeared from other laboratories (2, 3, 4). Several workers, however, who have employed blood acetone measurements to detect ketosis have reported good ketonemic responses in adrenalectomized rats (2–6). Neufeld and Collip, and Mirsky found a slightly higher average ketonemia in groups of injected adrenalectomized rats than in groups of injected control animals.

If one assumes that the urinary excretion of acetone bodies is reduced after adrenalectomy, two alternative causative mechanisms can be proposed. Either the blood acetone body response is diminished, or the renal threshold is elevated. In order to decide between these two alternatives, the present work was undertaken. The experiments have included a quantitation of the rise of blood acetone bodies after the injection of pituitary extract in adrenalectomized rats, and a determination of the effect of adrenalectomy on the renal threshold by simultaneous estimations of ketonemia and ketonuria. In previous work (7) it was found that normal rats have a renal threshold for acetone bodies at the level of approximately 25 mg.% in the blood.

In the present study a micro blood acetone technic was employed which had been designed for use in previous experiments on ketogenesis (7), and which in the hands of the author represents the only procedure which has allowed a reasonably dependable and quantitative evaluation of the ketosis induced by the injection of an extract. Because the method requires only 0.2 cc. of tail blood, it is possible to avoid killing the animal, hence blood and urine may be collected simultaneously during observations on the renal threshold.

METHODS

Two types of experiments were performed. The first consisted of a quantitative study of the ketonemic response of adrenalectomized rats to pituitary extract, while the second embodied a study of renal threshold in relation to adrenalectomy. In the first experiments, blood was taken from the tail of normal and adrenalectomized rats fasted 48 hours, both before, and 4 hours after an intraperitoneal injection of Burn and Ling extract (8). These samples were analyzed for blood acetone bodies by the method previously described (7). The extract was given in doses of varying amounts

[1] A preliminary report was presented before the American Physiological Society, Toronto, Canada, April, 1939.

so that response curves could be established for both the control and operated animals.

Rats which were used for the observations on renal threshold were fasted for 24 hours and then injected subcutaneously with 0.5 cc. of Burn and Ling extract (1.3 mg. of nitrogen per cc.). A sample of tail blood was taken 4 hours after injection, and the urine which was collected for 24 hours after injection was analyzed for acetone bodies by the method of Van Slyke (9). The actual peak in the rise in blood acetone bodies is not reached until 8 to 10 hours after injection, but experience has shown that the content in the blood at the end of 4 hours is sufficiently near the ultimate high point so that it may be used as a satisfactory index of the degree of ketonemic response.

The animals used in all experiments were of the Sprague-Dawley strain, and were males ranging in weight from 120 to 150 gm. Previous to fasting they had been maintained on a diet of Purina Dog Chow. Various groups of control and adrenalectomized rats were injected within a few weeks of each other in order to eliminate any difference that might be due to seasonal variations in sensitivity. The adrenalectomized animals were injected 4 to 13 days after a single stage operation. The time interval between operation and injection was deliberately made variable so that the effect of such a change on the reactivity of the animals could be observed. No adrenalectomized animals which failed to die with symptoms of adrenal insufficiency within 2 weeks after withdrawal of the 1% salt solution used to maintain them during the experiment, were retained in the series. All were examined at autopsy for remnants of adrenal tissue and for any complicating disease which might have caused death. In order to avoid the use of animals which were moribund at the time of injection, all rats dying within 24 hours after the conclusion of an experiment were excluded.

RESULTS

The ketonemic response of adrenalectomized rats is illustrated in figure 1 along with the normal curve for comparison. One sees that the adrenalectomized rats are

TABLE I. URINARY THRESHOLD FOR ACETONE BODIES

Blood acetone bodies	Urinary excretion, mg. per 24 hr. per rat			
	Normal rats		Adrenalectomized rats	
	No. in series		No. in series	
5 to 9.9 mg.%			1	0.6 mg.
10 to 14.9 mg.%	7	2.0 mg.	10	0.6 mg.
15 to 19.9 mg.%	2	3.4 mg.	11	2.8 mg.
20 to 24.9 mg.%	11	5.6 mg.	6	12.1 mg.
25 to 29.9 mg.%	5	9.8 mg.	2	15.2 mg.
Above 30	5	14.0 mg.	1	23.0 mg.
Total	30		31	

clearly reactive although at each dose level the rise is consistently less than in the normal group. Although it can be presumed by inspection of the graph that the adrenalectomized rats are less sensitive than normal, a satisfactory statistical proof of a difference in sensitivity between the two series is difficult to make. A statistical comparison of any two single groups which represent a given dose level can be made with ease, however. For instance, the mean response of the adrenalectomized rats

receiving the 20γ dose was compared with the mean of the normal group receiving the same dose, and a similar comparison was made in the case of the rats which received 40γ. In each instance the difference between the means was statistically significant[2] (10). It is in this range of dosage that the normal curve approaches a straight line; hence it is this portion which is best adapted for a comparison of potencies. A rough approximation of the sensitivity of the adrenalectomized rats in relation to those of the normal group suggests a decline in reactivity to about one-third that of normal. It should be noted that the adrenalectomized rats are less responsive to the larger doses than to the moderate sized ones, although the normal

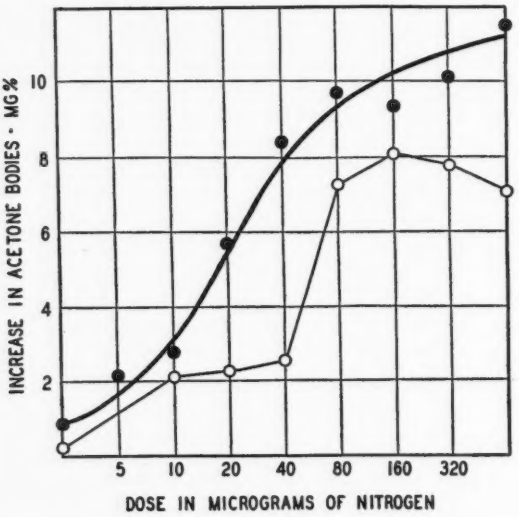

Fig. 1. Divisions along the abscissa are spaced in proportion to the logarithm of the dose, although dosage is expressed in actual γ of N given. The upper curve (black dots) represents the normal series. Each point is the average of from 5 to 10 determinations, and there are 64 rats in the entire series. No attempt has been made to construct a smooth curve from the plotted points representing the adrenalectomized rats (open circles), for the variability of response was considerably more marked than in the case of the normal rats. Each point represents the average of from 5 to 11 observations, and the series totals 56 rats.

curve continues to rise in the higher range of dosage. That the normal curve also ultimately declines is suggested by the finding of Fry that very large doses of Burn and Ling extract (3 cc.) failed to produce ketonuria and seemed toxic (1). The effect of increasing the time which was allowed to elapse after operation did not seem to be of any consequence inasmuch as the rats which were allowed to wait 7 to 13 days after operation were not preceptibly less responsive than those injected within 4 days.

From the data in table 1, it is apparent that the urinary threshold of the adrenalectomized rats showed no indication of being elevated. Most of the adrenalectomized animals exhibited no marked ketonuria, but when certain ones exceeded a blood level of approximately 25 mg.%, urinary excretion was sharply augmented, precisely as in unoperated rats. The 3 adrenalectomized rats in which the blood level exceeded

[2] At the 20γ dose: standard error of the difference of the means = 1.54, $t = 2.34$, $p = 0.03$; at 40γ standard error of the difference of the means = 1.70, $t = 3.47$, p is much less than 0.01. Each of the single groups comprised 10 rats.

25 mg.% excreted 17.5, 13.0 and 23.0 mg. of acetone bodies, respectively. Seventy per cent of the normal rats had blood levels in the range in which an augmented ketonuria would be expected, as compared to only 29% of the adrenalectomized rats. Fewer of the latter showed an augmented ketonuria because there were fewer which attained a sufficiently high blood level to exceed the renal threshold.

It is of importance to examine the effect of adrenalectomy on the ketosis of fasting without the added stimulus of injected pituitary substance. The average blood acetone body content of a group of 105 normal rats after 48 hours of fasting was 11.6 mg.%, and of 56 adrenalectomized rats 9.9 mg.% (standard error of the difference $=0.73$, $p=0.02$). The difference is small but there are 98 chances in 100 that it is significant. Inasmuch as a level of 1.5 mg.% is the maximum obtained in fed rats by the method which was used, it is evident that the adrenalectomized animals did not lose their susceptibility to the ketosis of fasting, although they did differ from normal in that their ketonemia was somewhat reduced. This is in opposition to the work of Thaddea and Kühn who report having found a higher blood level in adrenalectomized than in normal rats (11). Neufeld and Collip (3) obtained higher blood levels than normal in adrenalectomized rats early in a period of fasting, but very low values later in the fast.

Further evidence of a reduced response on the part of adrenalectomized rats to pituitary extract may be secured by analysis of the data obtained in the experiments on renal threshold. The acetone bodies in the blood of the 30 rats in the normal group 4 hours after injection averaged 23.2 mg.%, while the figure for the 31 adrenalectomized rats was 18.0 mg.% (standard error of the difference $=1.96$; $p=$ less than 0.01). The difference in sensitivity is clearly a significant one.

DISCUSSION

The results which have been presented are in accord with those of MacKay and Barnes (2) Neufeld and Collip (3), Mirsky (4), Houssay and Rietti (5) and MacKay and Wick (6) insofar as they indicate that adrenalectomy does not prevent a ketonemic response to anterior pituitary extract. It would therefore appear that the process does not depend entirely on an adrenal mediation. In this connection it should be noted that adrenotropic fractions of pituitary extract have not been found to possess ketogenic properties (7, 12, 13).

Our results do not confirm the findings of Houssay and Rietti (5) that rats gradually lose their reactivity 7 to 14 days after operation. If there were such a loss of sensitivity at remote postoperative intervals, one might attribute it to a gradual loss of existing presecreted cortical hormone from the tissues and, although the cortex may not mediate ketogenic activity by a sudden discharge of cortical hormone in response to pituitary stimulation, nevertheless the coexistence of the hormone might be necessary for reactivity. In view of the short survival time after adrenalectomy, it is unlikely that appreciable amounts of the hormone remain in the organism 7 to 13 days after operation. Hence it seems reasonable to conclude from our positive responses obtained after this interval that the presence of the adrenal cortical hormone is not necessary for reactivity to pituitary extract. The failure of Houssay and Rietti to obtain a response during the later postoperative intervals may be due to the fact that their rats at this time were possibly in poor condition. We have found that rats on the verge of collapse are unresponsive. An equally good explanation of their negative results is their use of the unfasted rat, a test object which we have found very much less sensitive to anterior pituitary extract than the fasted animal.

That adrenalectomy reduces the ketonemic response of rats under the conditions

of these experiments is discernible not only in the assay curves themselves, but also in the lower average acetone body level of the adrenalectomized group used for threshold studies, and in the fewer number of these rats which exceed the renal threshold level. It should be noted that the rats in the latter group were without food for only 24 hours and were therefore less likely to be suffering from any ill effects of the fast itself.

The reduced sensitivity which we have encountered in the adrenalectomized rat may be an indirect or non-specific effect dependent, perhaps, upon an abnormally weakened state of such a rat after a period of fasting, or it may be the result of an increased susceptibility of the operated rat to some inhibiting or toxic material in the extract. On the other hand, the cortical hormone may have a synergistic ketogenic function which is not indispensable inasmuch as some ketosis may be produced in its absence. A mechanism which entailed a partial mediation through the adrenal cortex might be postulated if one had evidence that the cortical hormone itself was ketogenic. MacKay and Barnes, in fact, have reported the production of ketonuria in rats with large doses of adrenal cortical extract (14). Their results are inconclusive, however, inasmuch as there was no correction for the comparatively large amount of ketone substance which is present in commercial cortical extracts, and which merely passes through the animal and out into the urine when the solution is injected[3] (15). Grollman (16) could not produce ketonuria with a purified cortical extract.

The present work indicates that there is no elevation of the renal threshold after adrenalectomy. In order to establish the existence of a renal threshold it is necessary to demonstrate that a sharp rise in urinary excretion occurs if a certain critical blood level is exceeded. Thus, it was previously found in the normal rat that blood acetone body concentration could vary from 1 to approximately 25 mg.% without any appreciably increased excretion, however, blood levels above the general range of 25 mg.% resulted in a distinctly augmented ketonuria (7). The present findings confirm those which were previously reported, and indicate that the threshold after adrenalectomy shows no tendency to be displaced above normal.

Neufeld and Collip (3), Mirsky (4), and MacKay and Wick (6) have suggested that the renal threshold is elevated in the adrenalectomized rat. Examination of the data of MacKay and Wick reveals no serious discrepancy with our own results and conclusions. If one takes their observations in which a blood sample was obtained after 24 hours of urine collection and superimposes them on a blood-urine scatter diagram of our own previous and present data, it is seen that both their normal and adrenalectomized animals fall within the same general range on the graph with no indication that those after adrenalectomy reach an excessively high blood level without an attendant ketonuria. Mirsky's failure to find augmentation of urinary excretion in adrenalectomized rats is probably due to the rather early and brief period of urine collection that he employed (the first 8 hours after injection). It has been our experience that a great proportion, if not the larger proportion of urinary excretion, occurs during the second half of the 24 hours after injection. That the ketonuria of adrenalectomized rats may be of substantial magnitude is indicated by the recent report of Harrison and Long (17). Mirsky's higher average blood level of acetone bodies in the adrenalectomized group could be related to the younger average age of these animals.

In experiments involving an estimate of the ketogenic response to an extract, the contradictory results of various investigators are not entirely unexpected. Not only

[3] A determination of the ketone content of an extract similar to that used by MacKay and Barnes indicated the presence of 2.1 mg. per cc. expressed as acetone. The Van Slyke method was used for the determination.

is there a fair sized variation in response from rat to rat after a given injection, but there is the added complication of the enormously variable response to fasting alone. For example, we have encountered blood acetone readings in fasting rats (of the same age and sex) scattered over the entire range from 1.5 mg.% to 30.0 mg.%, It was to eliminate the unpredictable influence of fasting that the present micro blood acetone method was devised.

SUMMARY

Adrenalectomy did not prevent the ketonemia which may be induced by anterior pituitary extract in fasted rats, although the response was reduced. There were fewer adrenalectomized animals than normal which manifested a rise in blood acetone bodies sufficient to exceed the renal threshold. Hence there were fewer in the operated series which showed ketonuria. No evidence was found for the existence of an elevated renal threshold for acetone bodies after adrenalectomy.

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EDITORIAL ARTICLE

THE ENDOCRINE LITERATURE OF 1939

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THE DIFFICULTIES of keeping up with medical literature in general have been presented by numerous persons on various occasions, never more aptly, perhaps, than by Sir Robert Hutchison.[2]

With more than 5000 medical journals to consider, certainly no single person can hope to do more than keep aware of the literature of his own specialty, but even the specialist is confronted with a problem too vast for his individual attention, and must either blissfully disregard it or set about to conquer it. To quote Sir Robert, " . . . Neglect of the previous literature must necessarily involve much needless repetition of effort and searching for that which is found, besides making it difficult for the investigator to see his subject in correct focus. The case of those, and they are the great majority of us, who are not investigators but who read only to learn and keep abreast of current knowledge, is equally hard . . . yet current medical literature cannot be permanently ignored with impunity."

The literature of endocrinology furnishes an outstanding example of the problems involved in making available the written evidence of man's rapidly and continuously increasing knowledge. While the subject is specialized in the sense that the study is focused on a few glands and their secretions, the specialization ends with their enumeration, for, if the literature of the subject bears veracious witness, there is scarcely an organ or function of the human or animal body which is not controlled or affected by the secretions of these glands. The literature is in the same manner specialized and diffused; concerned primarily with the endocrine system and its effects, it appears not only in journals devoted entirely to endocrinology but also in journals specializing in such diverse subjects, among others, as cancer, children's diseases, veterinary medicine, psychology and psychiatry, pathology, immunology, biochemistry, histology, dairy science and gynecology.

As a preliminary to a survey by one of us of the endocrine literature which appeared in 1938,[3] abstracts were gathered from 7 abstracting services[4] by members of the staff of the Institute of Experimental Biology. The references were later analyzed to identify the journals from which they were taken. More than two hundred journals appeared in this list, of which about eighty-five had published three or more papers relative to the subject during 1938, and only fifteen of which were journals concerned extensively with the literature of endocrinology. This study was greatly hampered by the time lag between the appearance of the original article

[1] Chairman, American Library Association. Serials Section. Committee on Indexing and Abstracting.

[2] HUTCHISON, SIR ROBERT: The Lloyd Roberts lecture delivered before the Medical Society of London on November 16, 1939; *Lancet*, 2: 1059. 1939.

[3] EVANS, HERBERT M.: Endocrine Glands: Gonads, Pituitary and Adrenals. *Ann. Rev. Physiol.* 1: 577. 1939.

[4] *Berichte über die gesamte physiologie; Berichte über die wissenschaftliche Biologie; Chemical Abstracts; Biological Abstracts; British Chemical and Physiological Abstracts;* ENDOCRINOLOGY abstracts; *Nutrition Abstracts and Reviews.*

and its abstract in a review journal, which, as will be shown, is almost never less than four months, and is usually more than six months and may be two years.

As an outgrowth of this survey, the Institute of Experimental Biology of the University of California undertook to compile an actually current monthly index of the appropriate articles appearing in about two hundred and twenty-five journals which regularly publish articles of interest to endocrinologists.

The work of indexing is so planned that the latest numbers of these journals are indexed as they are received in the University of California library and in the library of the University's Medical School, so that by the end of every month, the index is complete for all journals received during that month; i.e., by August 28, all journals received during August are analyzed and citations filed under the proper subject heading.

Arranged under rather broad subject headings, the index furnishes a quick survey of the articles appearing in the latest numbers of the most important journals concerned with the subject.

At the end of a year's work on the index, an analysis has been made of the problems involved in keeping up with endocrine literature, and the present paper sets forth the results of this study.

During the year 1939, 2807 papers of endocrine interest were selected from 221 journals. The number of papers appearing in each journal varied from 1 to 243. Eight journals published a total of 965 papers or 34% of the whole; 38 others contributed 1014 papers or 37%; while the other 29% were scattered about in 175 journals publishing from 1 to 12 papers apiece. Seventy-four journals published only one paper each; 101 journals published from 2 to 12 papers each, (a total of 754); 21 journals published 410 papers, from 13 to 25 papers apiece; 17 journals published from 26 to 50 papers each, making a total of 604; one journal published 52, one 54, and one 66 papers; two journals published 87 papers each; and the remaining three journals published 165, 211 and 243 papers respectively. A list of the 46 journals in the highest categories follows.

LIST OF JOURNALS PUBLISHING FROM 13 TO 300 ARTICLES ON ENDOCRINOLOGICAL SUBJECTS, 1939

One hundred to three hundred articles. ENDOCRINOLOGY (243)[5]; Societe de Biologie, Comptes Rendus (211); Society for Experimental Biology and Medicine, Proceedings (165).

Fifty to one hundred articles. American Journal of Physiology (87); Klinische Wochenschrift (87); American Journal of Obstetrics and Gynecology (66); Lancet (54); Journal of the American Medical Association (52).

Twenty-five to fifty articles. Anatomical Record (47); Zentralblatt für Gynäkologie (45); La Presse Médicale (43); Deutsche medizinische Wochenschrift (42); Helvetica chimica acta (42); Folia endocrinologica japonica (37); Acta brevia Neerlandica (36); Münchener medizinische Wochenschrift (35); British Medical Journal (34); Journal of Endocrinology (34, in three numbers only); Archiv für Gynäkologie (33); Nature (33); Journal of Biological Chemistry (31); Journal of Physiology (30); Journal of Laboratory and Clinical Medicine (28); American Journal of Cancer (27); Schweizerische medizinische Wochenschrift (27).

Thirteen to twenty-four articles. Societa Italiana Di Biologia Sperimentale, Bolletino (24); American Journal of the Medical Sciences (23); Archives internationales de pharmacodynamie et de thérapie (23); Biochemical Journal (23); Journal of Clinical Investigation (23); Berichte der deutschen chemischen Gesellschaft (22); Science (22); Surgery, Gynecology and Obstetrics (22); Proceedings of the Staff Meetings of the Mayo Clinic (21); Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Phar-

[5] Numbers in parentheses indicate the number of papers published in 1939.

makologie (19); Annales d'anatomie pathologique (18); Chosen Medical Association Journal (18); Journal of Pharmacology and Experimental Therapeutics (18); American Journal of Psychiatry (17); Archives of Pathology (17); Journal of Urology (17); Wiener klinische Wochenschrift (17); Annals of Internal Medicine (15); Zeitschrift für die gesamte experimentalle Medizin (15); Acta medica Scandinavica (13); Revista de la Sociedad argentina de biología y sus filiales la Sociedad de biología de Rosario y la Sociedad de biología de Cordoba (13).

An analysis of the interest in the individual glands is possible by a count of the papers appearing about each, and table 1 indicates the numerical and relative interest value of all the papers published during the year in these 221 journals.

It is apparent that even the individual endocrinologist can not hope to pursue

TABLE 1

	No. of endocrine papers published by kind and 'interest value' (221 journals)		Relation of papers abtracted in 1939 to those published in 221 journals	
	Total papers published in 1939	Interest value, in percentage	Papers abstracted in 1939	Percentage abstracted
Adrenals	249	8.9	142	57
Gonads				
Female reproductive system	635	22.7	274	43
Male reproductive system	239	8.6	102	43
Mammary glands	61	2.1	17	28
General and/or mixed papers	99	3.5	60	66
Pancreas	339	12.1	150	44
Parathyroid	40	1.3	23	57
Pineal	8	0.3	5	62
Pituitary				
Anterior	216	7.8	125	57
Posterior	56	2.0	37	66
General	51	1.8	24	47
Spleen	17	0.6	6	35
Thymus	26	0.9	5	19
Thyroid	201	7.1	78	38
General	88	3.1	32	35
Glandular interrelationships	378	13.5	221	58
Metabolic interrelationships	79	2.8	45	57
Vitamin-Endocrine Interrelationships	25	0.9	16	64
Total	2807	100%	1362	48%

both his work and the copious literature of his field, and is compelled to depend to a large extent upon the review journals for his knowledge of what is being done outside the boundaries of his own experience.

During the period of this study, the seven abstract and review journals previously mentioned were checked regularly and of the 2807 published papers, only 1362 were found to have been abstracted by the end of the year.

Table 1 was prepared to show the relation of the total of papers abstracted to the total number of papers published. The relative amount of interest in each gland is also apparent in the number of papers appearing about each; but the percentage of abstracting cannot be accepted as a valid test of relative interest in any particular gland, since the date of publication of the article and the time lag in abstracting might seriously affect the percentages if they could be analyzed that closely.

Although only 1362 papers were abstracted, they appeared in the abstracting services 2310 times, for only 724 papers were abstracted in one service only. A total

of 402 were abstracted in two services; 184 in three services; 36 in four services; 10 in five services and 6 in six services.

An analysis of the individual abstracting services will indicate their relative efficiency. Table 2 indicates the number of papers abstracted by each service and the relation to the total number of papers published as well as to the total number of papers abstracted. The abstracting service appearing in ENDOCRINOLOGY is analyzed more minutely in table 4.

TABLE 2. EFFICIENCY OF ABSTRACT SERVICES

	No. of papers abstracted	Percentage of	
		Total papers abstracted	Total papers published
Biological Abstracts	408	30	14
Berichte über die gesamte Physiologie	111	8	4
Berichte über die wissenschaftliche Biologie	47	3	1.6
British Chemical and Physiological Abstracts	587	43	20
Chemical Abstracts	905	66	32
ENDOCRINOLOGY[1]	761	56	27
Nutrition Abstracts	74	5	2.6

[1] See table 3 for more detailed analysis.

A study of the first dates of appearance of 1939 papers in these abstract journals brings to light the following comparisons.

Biological Abstracts: 25 abstracts were published from January to March; the other 385 abstracts were distributed from April to December.

Berichte über die gesamte Physiologie: 1 abstract appeared in April, 9 in May, the rest from August to December.

Berichte über die wissenschaftliche Biologie: 4 abstracts appeared in May, the rest from July to December.

British Chemical and Physiological Abstracts: none appeared earlier than June; rather well distributed from June to December.

Chemical Abstracts: 3 were published in March, 16 in April; the rest were distributed from May to December.

Nutrition Abstracts and Reviews: 26 appeared in July, 49 in October.

It is readily apparent that the shortest time lag for the bulk of the abstracting is 4 months, and the average time lag is 6 months.

So far we have been considering the whole scope of endocrine literature. As Sir Robert has pointed out, not all individuals suffer from that insidious bibliophilism which makes it possible to endure the problems connected with exhaustive research. There are many physicians who are not within the magic circle of a large medical or university library, and who are dependent upon a few journals for their contact with current literature. An analysis of one endocrine journal will be helpful in seeing how adequately or inadequately they are kept up-to-date in this specialty. Accordingly, the Journal, ENDOCRINOLOGY has been analyzed to determine how effectively it deals with the whole field of endocrine literature.

During 1939, ENDOCRINOLOGY published 243 papers or 8.6% of all the papers published in the 221 journals surveyed. Table 4 shows the numerical and percentage relations between the whole number of papers published and those published by ENDOCRINOLOGY. The 'interest value' of each gland as indicated by the number of papers published about each is shown by table 3, which also indicates the relation

between the interest value of all the papers published and those published in ENDOCRINOLOGY.

Aside from the original papers published, the section in ENDOCRINOLOGY devoted to abstracts of current literature presents the following picture.

Of all the services studied, it is the only one entirely devoted to the specialized field under consideration. During the year 1939, it published a total of 980 abstracts; of this number 514 were abstracts of papers which were published in 1938; 219 were advance abstracts of papers later to appear in full form in the same journal (of these, 7 were inserted twice); 247 only were abstracts of 1939 papers from other sources. Considering the total number of abstracts from other journals (761) as an average

TABLE 3

	Comparison of papers appearing in ENDOCRINOLOGY with all papers published			'Interest value' of papers published by ENDOCRINOLOGY compared to 'interest value' of total papers published	
	Total papers published	Papers published by ENDOCRINOLOGY	Percentage of total papers published	Interest value expressed in percentage	Percentage of interest of total papers published
Adrenals	249	18	7.2	7.4	8.9
Gonads					
Female reproductive system	635	61	9.6	25.2	22.7
Male reproductive system	239	33	13.8	13.7	8.6
Mammary glands	61				2.1
General and/or mixed papers	99	9	9.0	3.7	3.5
Pancreas	339	18	5.3	7.4	12.1
Parathyroid	40	4	10.0	1.6	1.3
Pineal	8	3	37.5	1.2	0.3
Pituitary					
Anterior	216	40	18.5	16.5	7.8
Posterior	56				2.0
General	51	4	7.8	1.6	1.8
Spleen	17				0.6
Thymus	26				0.9
Thyroid	201	17	8.4	7.0	7.1
General	88	9	10.2	3.7	3.1
Glandular interrelationships	378	21	5.5	8.6	13.5
Metabolic interrelationships	79	5	6.3	2.0	2.8
Vitamin-endocrine interrelationships	25	1	4.0	0.4	0.9
Total	2807	243	8.6	100%	100%

yearly total of abstracts, the relation of abstracts to the total number of papers published is 27%, and the relation to the total number of papers abstracted is 56%.

Since the primary purpose of abstracting is to describe the scope of papers presumably not otherwise available, the advisability of publishing advance abstracts of papers later to appear in the same journal may be questioned, especially if they are published at the expense of omitting abstracts of papers from other sources.

Table 4 is a detailed break-down by subject and time lag of the papers abstracted by this Journal. It is to be noted that although one 1939 paper was abstracted in April, no others appeared until July—in effect, the time lag between publication date and date of abstracting is 6 months. That the actual lag is greater is shown by the fact that many 1938 papers were not abstracted until November 1939. In fact,

of the total number of 1938 abstracts appearing during 1939, 192 or 37% appeared in July or later—from 7 to 11 months after the calendar year had sunk into oblivion.

TABLE 4. ABSTRACTS OF CURRENT LITERATURE (ENDOCRINOLOGY): ANALYSIS BY QUANTITY, DATE OF ABSTRACT IN RELATION TO PUBLICATION DATE OF ARTICLES, AND 'INTEREST VALUE' OF INDIVIDUAL GLANDS

Advance Abstracts for papers to appear in ENDOCRINOLOGY	219
Abstracts of papers which had appeared in other journals in 1938	514
Adrenals: Jan., 29; April, 8; July, 5; Aug., 8; Sept., 2; Nov., 10: total 62.	
Gonads: Jan., 7; Feb., 23; March, 37; April, 11; July, 11; Sept., 5; Oct., 21; Nov., 47: Total 162.	
Pituitary: Feb., 15; March, 14; April, 1; June, 105; July, 8; Oct., 5; Nov., 10: total 158.	
Pancreas: Feb., 7; March, 9; April, 4; July, 4; Oct., 3; Nov., 12: total 39.	
Parathyroid: Feb., 3; March, 4; July, 1; Oct., 2; Nov., 4: total 14.	
Thymus: Feb., 8; July, 3; Nov., 2: total 13.	
Thyroid: Feb., 5; March, 11; July, 5; Oct., 9; Nov., 14: total 44.	
General: Jan., 4; April, 9; Sept., 7; Nov., 2: total 22.	
Abstracts of papers which appeared in other journals during 1939	247
Adrenals: April, 1; Aug., 3; Sept., 3; Nov., 18: total 25.	
Gonads: Sept., 6; Oct., 15; Nov., 33; Dec., 56: total 110.	
Pituitary: July, 2; Oct., 7; Nov., 21; Dec., 14: total 44.	
Pancreas: July, 1; Oct., 5; Nov., 14: total 20.	
Parathyroid: July, 1; Oct., 4; Nov., 6: total 11.	
Thymus: July, 1; Nov., 1: total, 2.	
Thyroid: July, 1; Oct., 3; Nov., 15: total 19.	
General: Sept., 3; Nov., 13: total 16.	

So far this study has not considered the value of the papers published aside from a numerical relative interest value in the various aspects of the subject, nor is it proposed so to do. To propose fewer and better journals and a reduction in quantity and betterment in literary style and content of the papers themselves implies an unheard of self-restraint on the part of authors, or a publishing and editorial dictatorship as yet impossible to achieve. There are methods, however, of keeping the endocrinologist abreast of the literature without the almost ruinous cost of abstracting every paper that appears in this field.

The Utopian spoonfeeding solution implies, of course, several dictatorial mandates (some of which have already been recommended in other places and by other people)

(*a*), No paper should be accepted for publication in any scientific or medical journal unless accompanied by an abstract.

(*b*), These abstracts, together with the date of publication, page references, etc., should be turned over immediately upon publication to a central abstracting service; (supported by contributions from dues from society memberships, subscriptions from libraries, etc.).

(*c*), The abstracting service should publish monthly in a subject list the abstracts received that month, cutting down the time lag until it is only from about 6 weeks to 2 months.

(*d*), Copies of this abstracting service should be furnished free of charge to all members of contributing scientific and medical societies, and sold to other subscribers at rates calculated to stabilize the cost of publication.

The trouble with this Utopia is, of course, the fact that considerable financial support would be required to establish it, and further subvention would be necessary to maintain it.

Fortunately there are other ways to get around the difficulties outlined above. In view of the rapidly expanding cooperation developed by libraries, almost any person may, on payment of a small fee, obtain photostatic copies of individual papers

or whole journals; microfilm is making available whole runs of journals; papers can be abstracted by scholars and translators connected with universities; *if one knows what papers he wishes to consult*, one may, in many different ways obtain that information.

How can he know, however, what papers he wishes to consult, if he does not know what papers have been published? The authors of this paper propose to fill this gap by publishing the Monthly Index to Endocrine Literature, briefly described at the beginning of this paper. Already in operation for a year, it has been widely used locally by both experimentalists and practicing physicians; it offers a monthly list of about 250 articles arranged first by gland, then divided into experimental and clinical papers, with the more numerous experimental group further subdivided under anatomical, chemical and physiological headings. The proposed annual or biennial cumulation would include not only the same subject arrangement, but citations to abstracts published. Pending its publication, the compilers would be interested in receiving comments and suggestions and in answering questions about the Monthly Index to Endocrine Literature.

CLINICAL AND LABORATORY NOTES

EFFECT OF EXTRACTS OF GELDING URINE ON REPRODUCTIVE SYSTEM OF THE RAT*

Charles E. Rea†

THE PURPOSE of this paper is to describe effects of extracts of gelding urine on the reproductive system of the rat, especially on the prostate. Since castrates have an atrophic prostate it was thought that possibly some 'anti' substance may be excreted in the urine of the castrate (gelding) which, upon injection into another animal, would cause an atrophy or decrease in weight of the reproductive organs.

Method. Urine was obtained from a 9-year-old gelding at the Farm School, University of Minnesota. Four liters of this urine were extracted with an equal volume of ether, 3 times,

TABLE I. EFFECT OF EXTRACTS OF GELDING URINE ON REPRODUCTIVE ORGANS OF THE RAT

Extract	No. of rats	Ave. wt. of rat	Ave. wt. of prostate	Ave. wt. of genital organs	Genital Wt. / Body wt.	Prostate Wt. / Genital Wt.	Prostate Wt. / Body Wt.
		gm.	gm.	gm.	%	%	%
Gelding urine (1st series)	13	231	0.339	4.806	2.08	7.05	0.140
Gelding urine (2nd series)	12	233	0.477	5.454	2.34	8.75	0.205
Normal urine	11	254	0.679	5.900	2.32	11.53	0.267
Control (1st series)	12	232	2.419	5.001	2.16	8.34	0.180
Control (2nd series)	13	235	0.532	5.728	2.45	9.29	0.226

to remove the lipoid-soluble substances. One cubic centimeter of the resultant aqueous extract was injected subcutaneously into adult male rats weighing 180 to 200 gm. Injections were made every other day for a total of 15 injections. The animals were then allowed to rest for 4 weeks before being killed. The weights of the rat and the reproductive organs were recorded. Untreated rats and rats injected with urine, prepared in the same way, from normal adult human males served as controls. The rats were not litter mates.

Results. Comparing the results, the ratio of genital weight to body weight was about the same in the control rats, those injected with the extract of gelding urine, and the animals treated with the extract of normal urine. Likewise, there was no significant difference in the ratios prostatic weight to genital weight or prostatic weight to body weight between the control animals and those injected with the extract of gelding urine. For some reason, these latter ratios were much higher in the rats injected with extracts of normal urine, but this may be due to the size of the series.

It is possible that the strength of the extracts used in these experiments was not sufficient to affect the weights of the reproductive organs. However, the urine when concentrated 1½ or 2 times caused sloughs when injected into the rats.

Summary. Whole aqueous extracts of gelding urine failed to cause significant change in the weight of the organs of reproduction when injected into male rats.

* Supported by a grant from the Research Funds of the Graduate School of the University of Minnesota

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PREGNANCY DIAGNOSIS IN MARES BY COLORIMETRIC ESTIMATION OF ESTROGENS

Salomon Kober*

IN THE AUTUMN of 1937 we had an opportunity to determine the estrogen content of specimens of urine from more than 5000 mares, covered between April and August of that year. We used the method previously described by Kober.[1] In the following summer (1938) we asked the owners whether or not the mares had foaled. Reliable data were obtained in 3597 instances which enabled us to examine the value of the quantitative chemical estimation of estrogens in the diagnosis of pregnancy in mares.

Because previous work[2,3] had shown that a massive excretion of estrogens by the pregnant mare is not consistently present until this time, all our samples were collected 140 or more days after the last covering: 81.5% between the 145th and 150th day, 90.9% between the 145th and 155th, 0.8% between the 141st and 144th, and 8.3% between the 156th and 190th.

A negative diagnosis was made when the estrogen content was 0 to 2 mg./l. (the latter figure to allow for the experimental error inherent in the conditions of work[1]), a positive diagnosis was made when the content was more than 2 mg./l.

Taking then the 3597 sets of data: 2497 duly foaled or aborted, 2491 after a positive and only 6 after a negative diagnosis, giving the percentage of false negatives as 0.24. One thousand one hundred neither foaled nor were definitely known by the owners to have aborted, 1084 after a negative and 16 after a positive diagnosis. But of these 16, 10 might possibly have aborted unobserved and 7 of them had had a perceptible vaginal discharge (macerated fetus?). Moreover 3 owners are suspected of dishonesty in the consignment of urine as they possessed several animals and the urine was well paid for. Hence the percentage of false positives lies between 0.55 and 1.45 in this group, according to whether these doubtful cases are excluded or not. The total error over the two groups amounts to 0.61%, or 0.33% by corrective exclusion.

Compiling results from the diagnosis made, we get the following figures. Of 2507 cases "pregnant," 16 (or more probably 6) were not pregnant; error 0.64 (or more probably 0.24%). Of 1090 cases "non-pregnant," 6 were pregnant; error 0.55%.

Discussion. Numerically exact statements relating to pregnancy diagnosis in the mare are scarce in the literature. Svensson[4] applying the fluorescence reaction of Cuboni[5] to urine passed on or about the 140th day, gives the following figures: in 140 mares who later foaled, the reaction was negative 4 times, this relatively small material giving an error of 2.8% (as against our own 0.24%). Jastrzebski[6] by the same method, had no wrong diagnosis throughout a series of 57 pregnant mares tested after the 140th day, and only one error among 101 non-pregnant. But his material is insufficient to be comparable with our own. With the Aschheim-Zondek reaction in women the mean expected error is 3.4%.[7] In comparison with other pregnancy reactions, the quantitative determination of estrogen by chemical methods would appear to be very reliable.

Summary. The subsequent history of 3597 mares, whose urinary estrogen content had

* From the research-laboratories of N. V. Organon, Oss, Holland.

[1] KOBER, S.: *Biochem. J.* 32: 357. 1938.
[2] GLUD, PEDERSEN-BJERGAARD AND PORTMAN: *Endokrinologie* 13: 21. 1935.
[3] KOBER, S.: *Acta brev. Neerl.* 5: 34. 1935.
[4] SVENSSON: *Skand. Veterinaertidskr.* 26: 550. 1936.
[5] CUBONI: *Klin. Wchnschr.* 13: 302. 1934.
[6] JASTRZEBSKI: *Weterynaria Wspolczesna* 5: 132. 1938.
[7] MACK AND AGNEW: *Am. J. Obst. & Gynec.* 27: 232. 1934.

been estimated chemically between the 141st and 190th day after the last covering was obtained, in order to see whether they had foaled or not. Of these 2497 had foaled, but in 6 no estrogens (<2 mg./l.) were found in the urine. The error in this group amounts to 0.24%. A considerable quantity of estrogens was found in the urine of 16 mares of the 1100 which had remained barren. In 10 such instances there is a possibility that the animals were pregnant at the time of examination. The error in this group amounts therefore to 1.45 or possibly 0.55%. Where the verdict had been "pregnant," the error was 0.64 or more probably 0.24%; when the diagnosis was "non-pregnant," the error was 0.55%. The total percentage error over all the material presented lies between 0.61 and 0.33.

CURRENT ENDOCRINE LITERATURE

HANS O. HATERIUS, ABSTRACT EDITOR

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BOOK REVIEW

ENDOCRINE GYNECOLOGY. E. C. Hamblen. Charles C Thomas, Springfield, Illinois and Baltimore, Md. 1939. Pp. 453.

Any growing science has frequent need for reappraisal. Those sciences that are ancillary to medicine need, in addition, special focussing and interpretation to render available to the practitioner the findings of the specialists and the laboratory investigators, that are ready for productive employment. The middleman in such transactions has need for a special sort of competence. Ideally, he should be a catholic student of the literature in both its theoretical and applied phases; he should be a sound clinician in his own right; he should be an experimentalist and thus a critical judge of values; and he should have command of an attractive literary style. All these characteristics, the author possesses.

Unlike some books in which practicality is set up as the major aim, this one does not suffer from shallowness. Simplicity is not secured by omitting difficult essentials. The book does not offer an easy road to erudition but it does offer a rich opportunity to make contact with the more important and fresh discoveries of the recent past.

The book opens with a striking frontispiece, a reproduction of the Ducasse lunette of endocrine types. A foreword by Professor Collip comments upon the timeliness and conservatism of the work. The first section of the book proper, includes an interesting chronological summary of the outstanding developments in the field for the past two thousand years. The remainder of the section is devoted to a discussion of the various endocrine products that are of interest to the gynecologist.

Part 2 amounts to a discussion of functional gynecology, dealing as it does with the development and regression of the female organism and especially the endocrine factors in the evolution.

Part 3 deals with clinical endocrinology proper. An excellent chapter on diagnostic procedures is of special practical interest.

Then follow chapters on sex-endocrine syndromes, abnormalities of growth and development, uterine functions, menstrual disorders, sterility, and the climacteric. Excellent bibliographies are supplied for each section of the book.

The illustrations are notable for their adequacy and informative character. They are unique in that comparable items such as body size are drawn to uniform scale to permit ready comparison. The format of the book is attractive throughout.

Altogether it can be highly recommended not only to the general practitioner to whom it is explicitly addressed but to any gynecologist who does not have time to read voluminously in the original endocrine literature but who wants to keep freshly in touch with developments.

ADVANCE ABSTRACTS

AYCOCK, W. L. A subclinical endocrinopathy as a factor in autarceologic susceptibility to poliomyelitis. *To appear in this Journal.*

There is epidemiologic evidence that the virus of poliomyelitis is widespread, but that the occurrence of the paralytic disease in those exposed to the virus is limited and selective. Some of the selectivities indicate that the determining factor, as between clinical and subclinical poliomyelitis, resides in the host. Familial tendency suggests that this factor is inherent; seasonal and geographic selectivities indicates a physiologic rather than an anatomic character; association with a certain constitutional type points to endocrine dysfunction; and the suspected selectivity seen in the occurrence of poliomyelitis during pregnancy, in view of the implication of the nasal mucosa as the portal of entry of the virus, suggests that estrogenic substances are concerned. Two series of experiments are recorded. In the first set, the injection of estrogenic substance into castrate immature female monkeys enhanced resistance to intranasal instillation of the virus. The second series comprised a small number of comparative urinary estrogen assays on poliomyelitis patients and normal individuals. The results indicate a higher average excretion of estrogenic substance in the group of poliomyelitis cases. These observations suggest that autarceologic susceptibility to poliomyelitis may lie in some fault in the economy of estrogenic substance.

BLUMENTHAL, H. T. The mitotic count in the adrenal cortex of normal guinea pigs. *To appear in this Journal.*

The average number of mitoses in a section of adrenal gland of the guinea pig, 6μ thick, has been found to be 4.1. There is a strong probability that a gradual decrease in the mitotic proliferation takes place from embryonic life to the age of 2 years. No significant difference was observed between the mitotic counts in male and female guinea pigs. In a relatively small number of experiments the count was higher in animals kept at a temperature of 20°C. than in those kept at a temperature of 30°C.

BLUMENTHAL, H. T. The influence of time of feeding on the periodicity in activity in thyroid and adrenal gland of normal male guinea pigs. *To appear in this Journal.*

If guinea pigs are fed once in 24 hours, a rise in the mitotic activity of adrenal cortex and thyroid gland takes place, which as a rule reaches a maximum somewhere between the 4th and 12th hour following the feeding. This rise seems to occur irrespective of the time of day when the feeding takes place. It is probably due to metabolic processes which are initiated by the intake of food. There is no indication in these experiments that light plays a part in the determination of rhythmic mitotic activity; but further studies are necessary to determine whether it may not be a subsidiary factor.

BLUMENTHAL, H. T. The effect of fresh and experimentally modified anterior hypophysis of cattle on mitotic activity in the adrenal cortex of the guinea pig. *To appear in this Journal.*

Implantation of fresh cattle anterior hypophyseal lobes into immature female guinea pigs causes an increase in the number of mitoses in the adrenal cortex of these animals; injections of acid extract of cattle gland has a similar effect. Immersion of cattle hypophysis in 95% alcohol, acetone, glycerin or 50% urea before implantation results in an increase in mitotic activity, which is not as high as that observed after implantation of fresh gland; treatment with combinations of urea and glycerin give similar results. After immersion of cattle gland first in urea and then in 95% alcohol, or vice versa, there is either no or only a slight stimulation of mitotic proliferation in the adrenal cortex. Fifty per cent urea has been shown to extract a part of the hormone, which is the cause of increased mitotic proliferations; this extractive action can be diminished or perhaps prevented by first treating the cattle gland with acetone or saturated ammonium sulfate before immersing it in 50% urea. The mitotic activity in the cortex of the adrenal gland seems to be a much more sensitive indicator for the determination of the effect of certain hormones than the change in weight which takes place in the gland under the influence of various agents.

CANTILO, E. Successful responses in diabetes mellitus of the menopause produced by the antagonistic action of sex hormones toward pituitary activity. *To appear in this Journal.*

A group of 40 women in the menopause or the postmenopause presenting diabetes mellitus, formed the material for this study. All of the patients treated showed evidence of a disturbed hormonal balance characterized by hyperactivity of the pituitary, which should be attributed to the diminution of the gonadal activity. Successful responses in 100% of the reported patients, including some severe cases of diabetes with gly-

cosuria and ketonuria, emphasize the necessity of separating this special type of climacteric diabetes characterized in particular by a diminished carbohydrate tolerance, which differs in no essential from that of the Cushing's disease. While insulin treatment and diet were carried out in our patients without any other endocrine therapy, the disturbed carbohydrate metabolism remained almost unchanged. Successful responses were obtained only with estrin and progesterone in large doses. The condition of the patients after stopping treatment, showed the remarkable effects of this therapy. No implication is intended that our patients were genuinely cured, however, the marked inhibition exerted on the pituitary activity by the sex hormones justifies this therapy—the only treatment at present which produces a cured-like state, with sugar free urine and normal blood sugar level. The fact that a slow improvement of the diminished glucose tolerance has been observed in several cases, might be attributed to a persistent hyperactivity of the pituitary body. Following medication, normal levels in the carbohydrate tolerance should be obtained.

D'Amour, F. E., and M. C. D'Amour. A comparison of the international gonadotropin standards. *To appear in this Journal.*

International Standard Gonadotropin from pregnant mare serum was assayed by 5 methods: effects on weight of seminal vesicles, ovaries and uteri, degree of luteinization and estrous smear. A previous study of the International Standard Gonadotropin from pregnancy urine permits a comparison of the two gonadotropins. The conclusions reached are as follows: One I.U. of PMS is approximately equivalent to one I.U. of PU, the latter being slightly more active. At higher dosage levels the effects of equal doses are not the same, thus, 30 I.U. of PMS produces ovaries 6 times as large as 30 I.U. of PU. On the basis of simplicity, sensitivity and objectivity a combination of the uterine weight—estrous smear technics appears to be the most satisfactory assay method.

Danby, M. Formation and destruction of male hormone by surviving organs. *To appear in this Journal.*

Surviving testicles, when perfused with blood, produce comb growth promoting substances; probably testosterone is formed. After adding homologues of testosterone to the perfusion blood, these substances are converted by the testicle into substances with a stronger comb growth promoting effect. Testicle pulp kept in an incubator shows no increased amount of male hormone and is not able to transform homologues of testosterone. When livers and kidneys are perfused with blood containing male hormone, only a small part of the added substance is recovered after the experiment; pulped livers and kidneys show no influence on added male hormone.

Evans, H. M., and M. E. Simpson. Experimental superfecundity with pituitary gonadotropins. *To appear in this Journal.*

Anterior pituitary gonadotropin when administered to immature female rats at certain levels brings about the phenomenon of superovulation and the implantation of an unusually high number of ova. A large proportion of such implantations undergoes resorption so that at the end of gestation which is usually somewhat prolonged, fewer than normal young are born.

Hamblen, E. C., W. K. Cuyler and M. Baptist. Urinary androgens and uterine bleeding. *To appear in this Journal.*

The average urinary titers of androgens of 9 women were determined by the colorimetric technic of Oesting during bleeding and nonbleeding phases. Seven of the 9 showed significant decreases in these titers during bleeding. The average daily titer of the entire group during the nonbleeding phase was 72 I.U. and for the bleeding phase it was 50 I.U.

Hamblen, E. C., W. K. Cuyler and D. V. Hirst. Urinary excretion of pregnandiol-complex by males. I. Following its oral administration. *To appear in this Journal.*

Single oral doses of 10 and 30 mg. of sodium pregnandiol glucuronide were given 6 healthy males and their urinary excretion of this compound studied by the method of Venning. Within 3 to 5 days most subjects excreted in the urine from 30% to 50% of the substance ingested. One subject failed to excrete any of the pregnandiol-complex. Gastro-intestinal absorption and renal filtration of this compound are discussed.

Hamblen, E. C., W. K. Cuyler and D. V. Hirst. Urinary excretion of pregnandiol-complex by males. II. Following intramuscular administration of progesterone. *To appear in this Journal.*

Intramuscular injections of progesterone in oil, which varied as to frequency and amount from single injections of 20 mg. to 4 daily injections of 10 to 30 mg., were given 6 healthy males without any untoward symptoms. Urinary titrations following these treatments were made by Venning's method for sodium pregnandiol glucuronide. Amounts of the pregnandiol-complex, which ranged from 0% to 42.5% of the calculated theoretical yield, were recovered. Marked variations were observed to occur in the time of final disappearance of the pregnandiol-complex from the urine in relation to the last injection. Factors involved in the metabolism of progesterone by males are discussed. Possible applications of these data to problems of gynecic physiology and gynecologic therapy are suggested.

HILL, R. T., AND M. T. STRONG. Ovaries secrete male hormone. V. A comparison of some synthetic androgens with the naturally occurring ovarian androgen in mice. *To appear in this Journal.*

A comparative study is made of the growth curve obtained on castrate male mouse accessories by the use of ovarian androgens as obtained from ear grafts, and the injection of several synthetic androgens. The data presented point to the growth curve of accessories resulting from injections of testosterone propionate plus progynon-B as being essentially identical with that obtained as a result of grafted androgenic ovaries. The physiological similarity exists although the chemistry of the ovarian principle remains unknown.

MCCULLAGH, D. R., I. SCHNEIDER AND F. EMERY. Spectrophotometric studies of the m-dinitrobenzene reaction with sex steroids. *To appear in this Journal.*

Spectrophotometric curves have been run of the color produced by the reaction of m-dinitrobenzene and the following steroids: androsterone, dehydroandrosterone, estrone, androsterone benzoate, testosterone, methyl testosterone, cholestenone, pregneninolone, pregnenolone, alpha-estradiol and androstenediol. A similar study was made on extracts of human male blood, bovine testis tissue and normal human male urine. These curves could not be related to those observed using pure steroids. The curves for the 17-keto steroids were qualitatively and quantitatively almost identical. Similarly, the curves for the 3-keto steroids were all very similar but differed markedly from the 17-keto steroids. Pregnenolone, a 20-keto steroid, was again different in that it showed a similar reaction to the 17-keto steroids, but differed markedly in total transmission. The non-ketonic diols did not react with m-dinitrobenzene.

NEUFELD, A. H., S. M. SCOGGAN AND G. S. STEWART. The effect of pituitary preparations on the total body glycogen, water, nitrogen and fat of mice. *To appear in this Journal.*

The administration of certain anterior pituitary preparations to normal fasted mice (*dba*) resulted in a rise of total body glycogen, associated with a low fatty acids content. The distribution of fatty acids between the liver and the carcass suggests an increased fatty acid oxidation. The water retention observed may be accounted for by the antidiuretic potencies of the extracts. Total body nitrogen and its distribution between the liver and the carcass do not appear to be affected to any appreciable extent. It is suggested that the unusually high glycogen values observed in animals treated with the saline extract of fresh anterior lobes may be associated with the diabetogenic substance.

PAYNE, S., AND E. K. SHELTON Stilbestrol A synthetic estrogenic preparation *To appear in this Journal*

The synthetic drug, 4, 4 dihydroxy-A, B diethyl stilbene (stilbestrol) is a potent preparation, effective by mouth as well as by parenteral injection fulfilling and sometimes surpassing the therapeutic qualifications of the natural estrins A number of the untoward reactions herein and elsewhere reported may be the result of estrin overdosage Its potency, ease of administration and low cost make it conducive to therapeutic abuse. Extreme caution as regards its indiscriminate administration is warranted until after more intensive study of its toxicology

PREHEIM, D. V. Studies on thyroidectomized rats with special reference to lactation and growth. *To appear in this Journal.*

Female rats were thyroidectomized through 3 successive generations and bred to litter-mate males. The thyroids of these females and those of their progeny were treated with osmic acid to show the reticular apparatus of Golgi and studied histologically and cytologically. The growth curve of the litters was taken as a measure of lactation. Thyroidectomy in female white rats reduced the growth of their offspring 12.13%. Since the growth of young rats is entirely dependent on the maternal milk, it follows that lactation was in some way affected. The effects on lactation were not cumulative through 3 successive generations of thyroidectomy, nor could any constant histological or cytological changes be demonstrated in the thyroids. The gestation periods of the thyroidectomized animals were prolonged approximately 24 hours.

RAWSON, R. W., AND W. T. SALTER. Micro histometric assay of thyrotropic hormone in day-old chicks. *To appear in this Journal.*

The advantages of this assay are as follows: it can be performed on readily available biological material; it combines technics which have already been tested thoroughly; and it gives such high sensitivity that a numerical evaluation of thyrotropic activity can be had for concentration of thyrotropic hormone as low as those found in normal human urine. Furthermore, the method can easily be calibrated against known standard material by each investigator so that his results are readily interpretable elsewhere.

ROSEN, S., M. C. SHELESNYAK AND L. R. ZACHARIAS. Nasogenital relationship. II. Pseudopregnancy following extirpation of the sphenopalating ganglion in the rat. *To appear in this Journal.*

The sphenopalatine ganglia were removed by bilateral extirpation from a large group of adult female rats. This interruption in the non-olfactory

nerve supply to the nasal mucosa was followed by prolonged diestrus. Stimulation of the uteri during the prolonged leukocyte smear phase produced decidual growths.

RUDY, A. An unusual case of deficiency disease in a patient with diabetes mellitus. *To appear in this Journal.*

A case of deficiency disease in diabetes mellitus, not unlike pellagra, with neurological, skin and gastrointestinal manifestations simulating central nervous system lues and gastrointestinal malignancy. Its treatment is discussed.

SIEGLER, S. L. Further experiences with the hormone of pregnant mare serum. *To appear in this Journal.*

The hormone of pregnant mares' serum (gonadogen) was administered intramuscularly and intravenously, cyclicly and continuously, to 79 cases with various degrees of response. Complete biphasic ovarian response was assumed to have occurred if the endometrium revealed a secretory phase; if the vaginal cytology was premenstrual in character; if titration of the urine revealed the presence of sodium pregnandiol glucuronidate; if persistent cyclic bleeding followed cessation of treatment. This series included: 7 cases of primary amenorrhea, 2 of which gave positive results; 12 cases of secondary amenorrhea, 4 of which were positive; 19 cases of oligomenorrhea, 15 of which reacted favorably; 8 cases of anovulatory cyclic bleeding, 5 of which reacted favorably; 5 cases of hypermenorrhea, 4 of which gave positive response; and 28 cases of sterility, 11 of which resulted in pregnancy, 3 of these aborted, 5 delivered normally, and 3 have not as yet gone to term. This hormone of pregnant mares' serum seems to be efficacious only in those cases in which pituitary insufficiency is diagnosed, and in which the ovaries are receptive to this form of stimulation.

VAN WAGENEN, G., AND A. H. MORSE. Cyclic changes in the exteriorized uterus. *To appear in this Journal.*

Subsequent to the formation of a permanent utero-abdominal fistula in two monkeys there occurred a rhythmic extrusion and evagination of the uterine fundus with exposure of the endometrium. This excursion of the uterus when correlated with the appearance of vaginal bleeding coincided roughly with the latter half of the cycle, but usually there was an interval of 1 to 3 days before bleeding. About the middle of the cycle the skin which had closed over the entrance to the uterus ruptured and the uterus slowly pushed out taking 3 or 4 days to reach full extrusion. The return of the uterus was abrupt. When secretory endometrium was obtained by curettage it was always at a time when the uterus was extruded. However interval endometrium was sometimes found during this phase, indicating that the uterine protrusion was independent of any change which could be attributed to the presence of a corpus luteum.

VEST, S. A., J. E. DREW AND O. R. LANGWORTHY. Implantation of crystalline testosterone in the monkey. *To appear in this Journal.*

A comparison of the effects in the accessory genitalia of immature, castrate macaques was made following subcutaneous injections of testosterone propionate with those following implantations of pellets of crystalline testosterone. The histologic changes in the accessory genitalia following implantation were identical with those following injection. A slightly greater relative increase of weight seemed to occur in the cranial lobe as compared with the caudal lobe regardless of how the hormone was given. Taking the increase in weight of the accessory genitalia as a criterion, it was found under the experimental conditions that testosterone in the form of crystalline pellets produced a greater relative increase in weight. This was calculated in mg. of accessory genitalia tissue per mg. of absorbed hormone. These experiments seem to indicate that in the primate implantation of testosterone in the form of crystals may be a more efficient and more physiological method than that of injecting testosterone propionate in oil.

WEAVER, T. A., JR., AND P. C. BUCY. The anatomical relationships of the hypophysial stem and the median eminence. *To appear in this Journal.*

Blocks including the base of the hypothalamus with the hypophysis attached from 9 monkey and 4 cat brains were sectioned serially in either the sagittal or in the frontal plane and were stained by various methods to show neurons, neuroglial elements, nerve fibers and connective tissue. Microscopic study of these sections showed that the portion of the median eminence which lies posterior to the third ventricle is histologically closely related to the neurohypophysis, whereas the portion which lies anterior to the third ventricle is more closely related to the hypothalamus proper. It was shown also that the pars tuberalis is not always coexistent with the neurohypophysis. The significance of these anatomical facts to physiological experiments on the neurohypophysis were discussed with especial reference to the production of experimental diabetes insipidus.

ABSTRACTS OF ENDOCRINE LITERATURE

ADRENALS

COPE, O. AND SCHATZKI, R. Tumors of the adrenal glands. A modified air injection roentgen technic for demonstrating cortical and medullary tumors. *Arch. Int. Med.* 64: 1222. 1939.

Periadrenal insufflation of the air at present yields the most promisng diagnostic possibilities. In order to avoid complications, air is injected slowly, under low pressure, in small volume and into the lower rather than the upper portion of the renal space. The uses and limitations of the procedure as indicated by the results of 163 injections in 78 patients are discussed. It is concluded that this procedure can be performed on ambulatory patients and without fatality if the proper safeguards are employed. It is an important adjunct in the inclusion or exclusion of disease of the adrenal gland, either cortical or medullary. Not only can tumors be diagnosed earlier in the course of the disease but operative explorations of the adrenal glands can in certain cases be obviated. The mortality in the treatment of patients suspected of having disease of these glands will thus be lower.—I. B

DEL CASTILLO, E. B., R. FINOCHIETTO AND T. SCHLOSSBERG. Treatment of the syndrome of adrenal cortex hyperfunction (Le traitement du syndrome de l'hyperfonction cortico-surrenale). *Presse méd.* 47: 1018. 1939.

Four cases of 6 treated responded favorably to large doses of progynon B. All patients presented typical genito-adrenal syndromes.—A. E. M.

SPIEGEL, A. Masculinization of castrate guinea pigs by spontaneous tumors of the adrenal cortex (Auftreten von Adenomen der Nebennierenrinde mit vermännlichender Wirkung bei frühkastrierten Meerschweinschenmännchen). *Klin. Wchnschr.* 18: 1068. 1939.

Of 14 ♂ guinea pigs castrated shortly after birth, 3 showed a return of normal ♂ characteristics at ages from 2½ to 4 years. The entire ♂ tract partook of this return toward functional normality. Autopsy revealed no trace of testicular tissue, but did reveal large adrenal cortical tumors in each of the 3 animals. Autopsies on 100 other animals, of both sexes, from the same strain disclosed no adrenal tumors. The author raised the possibility that the early castration may have been related to the tumor development.—M. H. F.

THADDEA, S. Adrenal cortex and regulation of blood pressure (Nebennierenrinde und Blutdruckregulation). *Endokrinologie* 21: 338. 1939.

The lowered blood pressure of compensated Addison's disease is not influenced by specific hormone therapy, but in decompensated Addison's disease cortical hormone causes an elevation of the greatly lowered blood pressure. Addison patients showed disturbed blood pressure regulation with changes in body position, as well as a disturbed adrenalin blood pressure reaction, both of which are improved by treatment with cortical hormone or cystein.—W. J. A.

ENDOCRINE GENERAL

BLÖTNER, H. Alcohol tolerance tests in normal individuals and in patients with diabetes mellitus and diabetes insipidus. The effect of pituitrin, insulin, food and forced water on blood and urine alcohol levels after the ingestion of alcohol. *New England J. M.* 220: 283. 1939.

Alcohol tolerance tests in normal persons and in patients with diabetes insipidus or diabetes mellitus were made following ingestion of 0.6 cc. absolute alcohol per kg. body weight, and the effects of insulin, pituitrin, food and forced water on these tests were determined. Symptoms of intoxication appeared at blood and urine concentrations of below 63 mg.%. After a test meal the alcohol curves were slightly higher in diabetes insipidus patients than in normal persons. Pituitrin reduced blood and urine levels in both groups, yet it increased appreciably the symptoms of intoxication in the former. In diabetes mellitus patients blood and urine alcohol levels were appreciably higher than normal. Insulin had no effect on the alcohol curves or on symptoms. It appeared that alcohol is more harmful to the diabetic individual. Food ingestion before alcohol produced a striking decrease in blood and urine alcohol levels and in symptoms in diabetic patients. The diuresis of forced water intake in uncontrolled diabetes insipidus did not dilute the alcohol concentration in urine, but increased the total amount of alcohol excreted. Forcing water in the alcoholic patient produced no therapeutic effects.—Author's summary.

DAVIDSON. S. Infantilism in ulcerative colitis. *Arch. Int. Med.* 64: 1187. 1939.

Infantilism in chronic disease is characterized by retardation or absence of sexual development, usually accompanied by diminished growth. Although usually considered secondary to the underlying debilitating condition, both disorders in some cases have been considered to be due to a primary disease of the pituitary-hypothalamic complex. The author discusses briefly the literature on the subject and presents 3 cases of infantilism associated with chronic ulcerative colitis. There seems to be no previous mention of this association in the literature. The processes of

nutritional deficiencies in the chronic diarrheas are pointed out, and their rôle in the production of secondary infantilism is discussed. The rôle of other metabolic disturbances in chronic diseases is mentioned.—I. B.

DRAGSTEDT, L. R. The present status of lipocaic. *J.A.M.A.* 114: 29. 1940.

Evidence is summarized supporting the hormonal nature of this pancreatic extract, based upon the observations that (1) depancreatized dogs, fed mixed diets, were not restored to normal by insulin and pancreatic juice alone; the deficiency however was corrected by oral pancreatic tissue but not by feeding of other organs. (2) Extracts of pancreas were effective orally or subcutaneously in daily doses of 60 to 100 mg. dried substance, and since they were practically lecithin and choline free, these chemicals were not regarded as the effective agents. (3) Two types of fatty infiltration of liver occur in pancreatic diabetes, one due to inadequate insulin therapy, characterized by normal or high concentration of blood lipids and acidosis, and one due to lipocaic deficiency and characterized by low concentration of blood lipids, impaired liver function, decreased dextrose excretion and insulin sensitivity. This was relieved by lipocaic administration but not by insulin. (4) The more frequent occurrence of arteriosclerosis in depancreatized dogs suggests a possible relation of lipocaic to this disturbance in pancreatic diabetes and in diabetes mellitus.—H. O. H.

FISCHER, S. Treatment of schizophrenia with glandular extracts. *Arch. Neurol. & Psychiat.* 42: 644. 1939.

The author reiterates his earlier findings that schizophrenia is characterized by depression of the O_2 consumption rate and/or lowering of the specific dynamic reaction to protein. He deduced that the psychosis is due to deficient secretion of the anterior pituitary gland. Treatment of the disorder with pituitary extract and with x-ray or diathermy stimulation of the pituitary gave negative results but with a *PU* preparation (praehormone) excellent results were obtained. In 93 cases complete remission was obtained in 63% and significant improvement in an additional 21%.—R. G. H.

HOEN, A., H. LANGFELD AND C. OEHME. Relations of thyroid and adrenals (Über die Beziehungen zwischen Schilddrüse und Nebennieren). *Endokrinologie* 21: 305. 1939.

Continuing previous studies the authors administered desoxycorticosterone to guinea pigs. Rather large doses—4 to 6 mg. per da.—prevented the rise in total metabolism caused by administration of thyroxin. Adm. of desoxycorticosterone also prevented or hindered the hypertrophy of the adrenal cortex which is seen in experimental hyperthyroidism. The influence on the metabolism of lactoflavin and of cholesterol also was studied.—W. J. A.

POLONOVSKI, M., AND H. WAREMBOURG. The action of endocrine extracts on the intermediate products of carbohydrate metabolism in the plasma (Action des extraits endocriniennes sur les produits intermédiaires plasmatiques du métabolisme glucidique). *Bull. Acad. de méd., Paris* 120: 60. 1938.

Various extracts were tested in normal persons and on patients with cancer and diabetes. Glucose was determined and the "residual chromic index" (ICR) of the plasma, which latter measures the intermediate substances of carbohydrate metabolism. The index increased when glucolysis was deficient. Spleen and testicle extracts caused a decrease of glucose and ICR, similar to the action of insulin. Adrenalin, folliculin and posterior pituitary extract increased blood sugar and ICR. Thyroxin had no definite influence on the blood sugar but reduced the ICR. The extracts which decreased the ICR in cancer patients had also a tendency to inhibit tumor development.—A. E. M.

RICHARDSON, J. S. The effect of adrenal cortical extract on the basal metabolic rate in thyrotoxicosis. *Acta med. Scandinav.* 98: 581. 1939.

Seven patients with hyperthyroidism were treated with adrenal cortical extract (eucortone.) Two of these showed a fall in B.M.R. which persisted and 3 a fall which was not maintained but rose rapidly to the initial levels. Two patients showed no change, nor did the 3 subjects given control injections. The evidence confirms earlier investigations that adrenal extracts produce an effect on the B.M.R. No significant symptomatic improvement occurred so that at present the medication has little practical application.—J. M. L.

DRUMMOND, J. C., R. L. NOBLE AND M. D. WRIGHT. Studies on the relationship of vitamin E (tocopherols) to the endocrine system. *J. Endocrinol.* 1: 275. 1939.

Vitamin E had no effect on the reproductive organs of immature or hypophysectomized adult rats. The pituitary glands of E deficient ♂ rats contained an increased amount of gonadotropic hormone, those from E deficient ♀ rats showed a decreased amount of luteinizing hormone. Pregnant mare serum caused a normal response in the ovaries of E deficient female rats. In E deficient ♂ ♂ only the interstitial cells of the testis were stimulated, the tubules were unaffected. Hypophysectomy of E deficient rats caused a further

decline in weight of the testes. The evidence described does not support the suggestion that the effects of E deficiency are due to hormonal imbalance.—G. B.

KOTHE, H. The thyreo-suprarenal state (Der Status thyreo-suprarenalis—M. B. Schmidt). *Endokrinologie* 22: 229. 1939.

The case is presented of a 54-year-old woman who died with symptoms of Addison's disease. At autopsy and in the microscopic study changes were found in the thyroid and the suprarenals, characterized by replacement of the parenchyma by lymphoplasmacellular infiltration and sclerosis on the one hand and regenerative processes in both glands on the other hand. In the left suprarenal there was a primary hypo-or-aplasia and the thyroid gave the picture of colloid struma.—W. J. A.

NOVICK, R. G. The Laurence-Moon-Biedl syndrome. *Am. J. Psychiat.* 96: 717. 1939.

A case—said to be the first recorded in a psychotic subject—is described. The patient showed, in addition to the classical physical stigmata, emotional instability and evidences of autonomic derangement such as localized sweating and dermographia. The author is inclined to ascribe the disorder to a lesion in the central autonomic nervous system.—R. G. H.

GONADS

ABARBANEL, A. R. The treatment of afterpains and painful engorgement in the puerperium with testosterone propionate. *Am. J. Obst. & Gynec.* 38: 1043. 1939.

In 25 women the use of 10 mg. of testosterone propionate served to prevent afterpains in 22 cases. In a control group 76% required opiates for relief. In a series of 49 women with severe afterpains relief was obtained similarly. In 50 cases of severe painful engorgement of breasts 10 mg. doses gave relief in 92%, but did not inhibit lactation in women who continued nursing.—E. L. S.

ALLEN, E. Gynecological problems of the adolescent girl. *Am. J. Dis. Child.* 58: 162. 1939.

Endocrine substances offer great possibilities in the treatment of gynecological disorders during adolescence but the author is opposed to their indiscriminate use until more definite knowledge is obtainable. Complete physical examination is essential in cases of puberty bleeding. Determination of B.M.R. is essential and thyroid therapy may be beneficial even in the presence of a normal B.M.R. Pituitary and estrogenic preparations are not as efficient in his experience as in those of other investigators. Roentgen and radium therapy should not be used to stop the bleeding because of the dangers of permanent castration. The author favors rectal and even vaginal examination of these patients.—M. B. G.

BÜTTNER, W. The activity of estrogenic stilbene preparations in women (Über die Wirkung der östrogenen Stilbenpräparate bei der Frau). *Zentralbl. f. Gynäk.* 63: 1918. 1939.

This series of papers is in substantial agreement, reporting the clinical use of diethylstilbestrol, and its diacetate and dipropionate, orally and by injection in oil. Treatment led to prompt inhibition of lactation when 6 to 9 mg. doses were given in 1 day. Pruritis and kraurosis vulvae yielded to local or systemic therapy. Secondary amenorrhea was relieved by oral or injection therapy, and in several cases when progesterone was used in sequence, secretory endometrium was identified. Climacteric symptoms were readily brought under control. Dosage varied from 0.05 to 5.0 mg., usually 1.0 to 3.0 mg. Oral therapy was dependable. All authors agreed that there was some tendency to nausea, emesis, vague lassitude, but none found other toxic manifestation of note, nor was therapy often interrupted by these difficulties. Preissecker thinks drugs of the atropin type reduced these unpleasant symptoms, and suggested that raising the doses of stilbestrol slowly will also help. There are no reports of long continued treatment of climacteric women.—E. L. S.

BACHMAN, C. Photometric determinations of estrogens. I. A modified Kober reaction for determining the total estrogens in a mixture of estrogenic steroids. *J. Biol. Chem.* 131: 455. 1939.

Kober's reaction for the colorimetric determination of estrogens was modified by diluting the original reagents with an equal volume of water and running the reaction in a bath of chlorinated diphenyl at 150° instead of at 100° for 9.5 minutes. The absorption obtained at 520μ with equivalent amounts of estrone, estriol and α-estradiol became the same at this time interval and the relationship between absorption and concentration from 10 to 60γ was linear. Different batches of color reagent gave reproducible results. —J. M. L.

BACHMAN, C. Photometric determination of estrogens. II. A new color reaction for estriol. *J. Biol. Chem.* 131: 463. 1939.

A specific color reaction for estriol was produced by heating it with 2% sodium p-phenolsulfonate in 85% phosphoric acid at 150° for 9 minutes. This gave a pink color with maximal absorption at 540μ instead of at 520μ as with Kober's reagent. Estriol in the presence of estrone can be determined by obtaining values using both reagents.—J. M. L.

Browne, J. S. L., J. S. Henry and E. H. Venning. The significance of endocrine assays in threatened and habitual abortion. *Am. J. Obst. & Gynec.* 38: 927. 1939.

The authors discuss the endocrine factors involved in normal pregnancy. In 35 women with threatened abortion or histories of repeated abortions they report studies of chorionic gonadotropin, estrin, and pregnandiol output. Based on this they propose certain concepts about the endocrine factors responsible for abortion, and suggest the rationale for use of progesterone in those cases where pregnandiol excretion is low, or particularly for the "transition period" when the ovarian production of progesterone subsides to be replaced by the chorionic supply of this compound.—E. L. S.

Burpee, E. McK., M. Robinow and J. T. Leslie. Gonorrheal vaginitis in girls treated with estrone (theelin), fever and sulfanilamide. *Am. J. Dis. Child.* 57: 1. 1939.

In 112 cases of gonorrheal vaginitis in girls of all age groups up to puberty, diagnosis was established by vaginal smears and occasionally by cultures. Three methods of treatment were used: 1. Theelin, 2000 I.U., in oil, 3 times a week by hypodermic injection. 2. Fever, produced by intravenous injection of typhoid vaccine. Five children were treated in the Kettering hypertherm. 3. Sulfanilamide, administered orally in doses of 5 gr. per 20 lb. of body weight, 3 times a day for 2 days then ½ the amount for 5 or more days. Apparent cures following the intramuscular injection of theelin in oil were obtained in 41 of 47 cases (87%). In 5 of these (12%) there were recurrences. Five patients treated with theelin and 1% silver nitrate jelly were cured and did not have recurrences. Apparent cures following fever as the only method of treatment were obtained in 8 of 19 cases (42%). There was 1 recurrence (12.5%). In 6 of 7 patients treated with fever during theelin therapy apparent cures could be attributed to the fever. Apparent cures followed the oral administration of sulfanilamide as the only method of treatment in 11 of 22 cases (50%). One patient had a recurrence. Two patients were cured by a combined treatment with fever and with sulfanilamide. One of these had a recurrence. All cures due to sulfanilamide were obtained in less than two weeks. Increase of the dose, prolongation of the treatment and combination with fever therapy did not improve the results.—M. B. G.

Campbell, N. R., E. C. Dodds, W. Lawson and R. L. Noble. Biologic effects of the synthetic oestrogenic hexoestrol. *Lancet* 237: 312. 1939.

Doses of 0.15γ administered to oophorectomized rats gave an estrous response in 60% of the animals whereas a dose of 0.1γ gave a 20% response. Hexestrol sensitized the uterus of the oophorectomized rabbit to progesterone and stimulated the nipples and mammary glands. Reduction of body growth and atrophy of the gonads were noted when its use was prolonged in rats. Assayed by injection into oophorectomized rats hexestrol was found to be more active than stilbestrol, and both were 2 to 3 times more active than estrone. Stilbestrol and hexestrol showed approximately the same degree of activity when given by mouth as when applied intravaginally. —L. B. S.

Dorfman, R. I., J. W. Cook, and J. B. Hamilton. Conversion by the human of the testis hormone, testosterone, into the urinary androgen, androsterone. *J. Biol. Chem.* 130: 285. 1939.

Testosterone propionate was administered to 3 hypogonadal men either intramuscularly in daily injections of 20 mg. or orally in tablets containing 60 to 120 mg. The assays for androgenic material was performed on the comb of the day-old chick using 15 to 20 chicks for each assay. After the administration of testosterone, the excretion of androgens increased from 10 to 20 I.U. to 60 to 70 I.U. The collected urines in each case were pooled and separated into ketonic and non-ketonic fractions by the Girard-Sandulesco reagent. From the ketonic fraction androsterone was isolated. This conversion of testosterone into androsterone was interpreted as indirect evidence that in man testosterone may be the testis hormone.—J. M. L.

De Wit, J. J. D., and L. H. Bretschneider. A biological difference between estrone and stilbestrol (Über einen Wirkungsunterschied zwischen Stilboestrol und Follikelhormon). *Klin. Wchnschr.* 18: 1423. 1939.

In contrast to the marked effects obtained with natural estrogens, the addition of stilbestrol to the aquarium water up to lethal doses (200γ per 750 cc. of bath) did not stimulate any growth of the ovipositor of bitterlings.—M. H. F.

Grumbrecht, P. Resort to thyroid preparations for menstrual disturbances (Die Anwendung von Schilddrüsenpräsenpräparaten bei Periodstörungen). *Zentralbl. f. Gynäk.* 63: 1942. 1939.

Using infantile rats receiving a constant dose of gonadotropic sheep pituitary powder, the ovarian weights varied with increasing doses of thyroid, showing a maximum with 75γ dose. It is pointed out that thyroid sensitizes ovaries to the gonadotropic action of pituitary, and that both hypo- and hyperactivity of thyroid in the human will be followed by ovarian disturbances. A case in point is cited, with relief from frequent

menses by the use of iodine in thyrotoxicosis, relief from amenorrhea by thyroid in a woman with low B.M.R., and treatment of secondary amenorrhea with gonadotropic extract and thyroid simultaneously.—E. L. S.

HEARD, R. D. H., AND A. F. MCKAY. II. The isolation of a new androstanol-3(β)-one and of allopregnanol-3(β)-one-20 from the urine of pregnant mares. *J. Biol. Chem.* 131: 371. 1939.

From the neutral fraction of equine *PU* there was isolated a new saturated hydroxy ketone, $C_{19}H_{30}O_2$, m.p. 187–187.5, $[\alpha]_d^{24} -160°$, which was precipitated with digitonin and formed an oxime, m.p. 194–195°, and a benzoate, m.p. 206–208°. Oxidation with chromic anhydride in the cold yields a diketone, m.p. 157–158°, not identical with androstanedione-3.17. The parent hydrocarbon was obtained by Clemmensen reduction of the diketone and identified as androstane. The compound is therefore an androstanol-3(β)-one, and the carbonyl group is probably located at C_6, C_7 or C_{12}. The occurrence of allopregnanol-3(β)-one-20, previously reported by Marker et al. was confirmed—J. M. L.

HIRSCHMANN, H. Androgens from the urine of ovariectomized women. *J. Biol. Chem.* 130: 421. 1939.

From 250 l. of urine collected from women with both ovaries removed dehydroandrosterone was isolated by precipitation with digitonin. From the ketones which were not precipitated by digitonin androsterone and α-3-hydroxyeliocholanone-17 were isolated by adsorption on alumina. These substances are thought to be produced in the adrenal cortex.—J. M. L.

JACOBSON, E., J. E. LACKNER AND M. B. SINYKIN. Electrical and mechanical activity of the human nonpregnant uterus. *Am. J. Obst. & Gynec.* 38: 1008. 1939.

By use of delicate balloon and tambour sets and of indifferent electrode pairs greatly amplified, the authors study the mechanical and electrical activity of the uterus in several normal women and some patients. The activity varied somewhat with the time in the cycle. The effects of posterior pituitary and of aqueous extracts of corpus luteum were tested, indicating very prompt results in the direction of stimulation by pituitary, relaxation by corpus luteum.—E. L. S.

LI, C. H., M. E. SIMPSON AND H. M. EVANS. Action of ketene on gonadotropic hormones. *J. Biol. Chem.* 131: 259. 1939.

Pituitary gonadotropic hormones were dissolved in acetate buffer at pH 5.7 and treated with ketene up to 2 hours at room temperature. The *FSH* and the *ICSH* lost a large part of their activity after 30 minutes' treatment. Pregnant mare serum (*PMS*) gonadotropin showed a similar loss of potency. *PU* preparations showed a smaller loss in potency after 30 minutes' treatment but 95 per cent inactivation after 2 hours. These studies indicate that the activity of the pituitary hormones and *PMS* are dependent on the presence of free amino groups, but that the hormone from *PU* requires unchanged phenolic hydroxyl groups.—J. M. L.

LIPSCHÜTZ, A. Antitumorigenic action of progesterone. *Lancet* 237: 420. 1939.

Five castrate ♀ guinea pigs received subcutaneous injections of 80γ of estradiol 3 times weekly. Two of these animals were treated for 47 days and 3 for 90 days. All animals at autopsy revealed uterine or extra-uterine tumors. Five castrate ♀ ♀ were treated as above plus 12 mg. progesterone weekly. Two animals were killed after 47 days, and 3 after 90 days. One animal at autopsy showed both extra- and intra-uterine tumors, whereas the remaining 4 did not. These few experiments support the author's hypothesis that the development of uterine fibromyomas in women are due to the disturbance of the normal balance between follicular and luteal hormones; and that progesterone may prove a useful therapeutic agent against fibromyomas.—L. B. S.

LUBIN, S., AND R. WALTMAN. Results of attempted induction of labor with estrin. *Surg., Gynec. & Obst.* 69: 155. 1939.

In attempting the induction of labor, the authors used 1 to 6 intramuscular injections of estrin ranging from 10,000 to 350,000 I.U. in 27 multiparae, and 9 primiparae. It was found that the onset of labor bore no relationship to the dosage employed. The authors suggested the possibility that labor in the human being near or at term might be induced, and its duration shortened by the use of estrin.—L. B. S.

DE MARVAL, L. Acidosis of ovarian origin (Acidosis ovárica). *Semana méd.* 46: 566. 1939.

A case is reported presenting the symptoms of asthenia developing during 5 years and final aggravation with intense hyperpnea, suggesting acidosis. The patient showed adiposity of ovarian type, and during the last years had developed oligomenorrhea. The alkali reserve was 40 cc. CO_2, but otherwise laboratory tests were negative. Sodium bicarbonate gave prompt but temporary relief. After injection of 10,000 units progynon the alkali reserve increased to 50 and the symptoms subsided. Prolonged cessation of the hormone treatment caused return of the hyperpneic syndrome.—A. E. M.

NEUWEILER, W. Hippuric acid synthesis in normal pregnancy and the puerperium (Über die Hippursäure-Synthese in der normal Schwangerschaft und im Wochenbett). *Klin. Wchnschr.* 18: 1050. 1939.

The 1 and 3 hour excretion of hippuric acid was investigated after intravenous injection of 1.77 gm. of sodium benzoate. In 18 normal controls most of the excretion took place during the 1st hour (0.7 to 0.9 gm.). In 42 pregnant women not only was the excretion retarded, but even the 3-hour total was less than that of normal nonpregnant women. In postpartum women, the excretion during the 1st hour was much below normal but the 3-hour total approached normal values.—M. H. F.

ROCK, J., M. BARTLETT AND D. MATSON. The incidence of anovulatory menstruation among patients of low fertility. *Am. J. Obst. & Gynec.* 37: 3. 1939.

Endometrial biopsies were studied from 392 women complaining of sterility, whose menstrual histories were known. Of these 1.9% showed evidence of at least occasional flow from proliferating or hypoplastic endometrium, indicating anovulatory bleeding. Repeated biopsies suggested about 4% may be considered habitually anovulatory. A history of menstrual irregularities (scanty or excessive flow or disturbance of the rhythm of periods) was from 3 to 9 times as frequent in sterility patients showing anovulatory menstruation as in patients who ovulated. A history of such irregularities should arouse suspicion of anovulation and should indicate repeated biopsy studies. In over 400 biopsies showing secretory endometrium, only 4 did not flow in 16 days and 2 of these flowed in 20 days. It seems that practically all patients, even with a history of amenorrhea, once they do ovulate, will flow within 16 days unless conception occurs. Lengthening of the cycle is due to prolongation of the preovulation phase, not of the secretory phase.—Author's summary.

SALABER, J. A., AND E. B. DEL CASTILLO. The action of pregneninolon by oral administration on the human endometrium (Acción del pregneninolón sobre el endometrio humano. Suministrado por vía oral). *Semana méd.* 46: 769. 1939.

Oral application of pregneninolon or anhydro-oxy-progesterone produced in a case of secondary amenorrhea uterine bleeding from a proliferated endometrium. A second case was treated with ethinyl-estradiol by mouth preceding pregneninolon treatment. At the induced menstruation biopsy showed a secreting endometrium. It was not possible to obtain intracyclic bleeding in a normally menstruating woman.—A. E. M.

SIEGLER, S. L. AND M. J. FEIN. Studies in artificial ovulation with the hormone of pregnant mares' serum. *Am. J. Obst. & Gynec.* 38: 1021. 1939.

Patients who were to have laparotomies were given PMS (gonadogen), usually 60 U., intravenously, at timed intervals before obtaining ovarian and endometrial tissues. Of 30 trials 6 showed no evidence of ovarian response, 5 showed diffuse follicular stimulation, and 3 more showed in addition to this stimulation a corpus luteum too old to be attributed to the therapy. But in 16 women there were recent corpora lutea which the authors attribute to the therapy. They feel that this confirms earlier reports on the ability to initiate luteinization by this extract.—E. L. S.

SMITH, G. V., O. W. SMITH, M. N. HUFFMAN, S. A. THAYER, D. W. MACCORQUODALE AND E. A. DOISY. The isolation of dihydrotheelin from human pregnancy urine. *J. Biol. Chem.* 130: 431. 1939.

Urine collected from women before and during labor was hydrolyzed, extracted and separated into weakly and strongly acidic phenols by treatment with bicarbonate. Theelin was separated by carboxy methoxylamine and dihydrotheelin by precipitation with digitonin. The digitonide was decomposed and purified as the di-a-naphthoate. The m.p., microscopic appearance and analyses agreed with those of a known sample.—J. M. L.

STIEGERT, F. Thyroid, uterus and ovaries (their internal secretory interrelationships) (Schilddrüse, Uterus und Ovarium (ihre innersekretorischen Beziehungen zueinander)). *Zentralbl. f. Gynäk.* 63: 1938. 1939.

Recent reports by Grumbrecht and Loeser are discussed. In rats they reported that salpingectomy or hysterectomy interfered with an effect of estrogen on the thyroid, which could be replaced by introduction of estrogen into the uterine cavity, but not by injection of estrogen with or without the uterus. The author elaborates a mechanism for effects of ovary on uterus, in turn on the thyroid, and in turn on the ovary, as a consequence of which hysterectomy is followed by abnormal ovarian cyclic action and ultimately atresia of follicles in spite of pituitary stimulation. No human data are included, although clinical implications are suggested.—E. L. S.

BIRNBERG, C. H., L. KURZROK AND S. J. KLOR. Inhibition of lactation during the puerperium by testosterone propionate. *Am. J. Obst. & Gynec.* 39: 107. 1940.

Best results were obtained by use of doses of 25 to 50 mg., total of 125 to 150 mg., intramuscularly, at intervals from 4 hours to 12 hours. Favorable results were obtained in 49 of 56 trials. The opti-

mum time for therapy cannot be determined from the results.—E. L. S.

COLLINS, C. G., J. C. WEED, B. B. WEINSTEIN AND F. R. LOCK. Clinical experiences with stilbestrol (diethylstilbestrol). *Am. J. Obst. & Gynec.* 39: 117. 1940.

Symptomatic relief was secured in an encouraging degree for 19 women with spontaneous menopause, 16 surgical castrates, 13 cases which are designated "hypoestrinism," and 2 with senile vaginitis. The authors use 5 mg. doses by injection once or twice weekly at first, and then maintain results with 1 mg. once or twice weekly. They believe the oral dose is about 3 times as large as by injection. They advise use of sodium bicarbonate with oral therapy, and believe the mild toxic symptoms were due to gastric irritation alone. No cases were intolerant to the drug. There are no protocols by which to judge the evidences for the opinions.—E. L. S.

DEANESLY, RUTH. The uterus masculinus of the rabbit and its reactions to androgens and oestrogens. *J. Endocrinol.* 1: 300. 1939.

Injection of estrogens into rabbits caused conspicuous growth of the uterus masculinus but suppressed the growth of its epithelium. The epithelium became normal only when large amounts of methyl testosterone or testosterone were administered by subcutaneous implantation of tablets.—G. B.

EDSON, M., AND R. D. H. HEARD. Hydrolysis of the conjugated estrogens in the urine of pregnant mares. *J. Biol. Chem.* 130: 579. 1939.

The estrogens liberated by hydrolysis from pregnant mare urine with hydrochloric acid at pH 0.2 to 1.2 at room temperatures for periods up to 6 weeks were determined colorimetrically by the method of Cohen and Marrian. Maximum conversion to the physiologically active ether-soluble free hormones takes place when urine acidified to pH 0.4 to 0.7 is allowed to stand for at least 4 weeks. The same optimum conditions apply throughout gestation.—J. M. L.

EDWARDS, E. A., J. B. HAMILTON AND S. Q DUNTLEY. Testosterone propionate as a therapeutic agent in patients with organic disease of the peripheral vessels. *New England J. M.* 220: 865. 1939.

Testosterone propionate in peanut oil was injected intramuscularly 3 times a week in 4 men with peripheral arteriosclerosis and 3 men with thrombo-angiitis obliterans. Spectrophotometric measurements indicated that more arterial blood was present in the skin after treatment. There was an increase in systolic blood pressure of from 6 to 26 mm. in the patients showing hypotension. All patients showed an improvement in walking ability and in the signs of intermittent claudication.—J. M. L.

EMMENS, C. W. The duration of action of certain natural and synthetic oestrogens when administered orally or by injection. *J. Endocrinol.* 1: 142. 1939.

Estrification of natural and synthetic estrogens which results in prolongation of action by injection, does not have this effect when the compounds are given orally. The minimum effective doses of ester and parent substance when orally administered are similar. The action of diethylstilbestrol dimethyl ether is slightly more prolonged than that of diethylstilbestrol when given by mouth.—G. B.

EMMENS, C. W., AND A. S. PARKES. The effect of route of administration on the multiple activities of testosterone and methyl testosterone in different species. *J. Endocrinol.* 1: 323. 1939.

Testosterone is more active by injection than by mouth in tests for androgenic activity in the capon and castrated ♂ rat, in metrotrophic tests on spayed rats or immature rabbits, and in tests for progesterone-like activity in rabbits. Methyl testosterone is almost as active by mouth as by injection in causing progestational proliferation in rabbits and has a relatively greater efficiency by mouth in the other tests than has testosterone. It is more potent than testosterone as an androgen in rats, less potent in capons. The two substances are equal in their ability to cause uterine growth in spayed rats and immature rabbits.—G. B.

EMMENS, C. W., AND A. S. PARKES. Some biological properties of anhydro-hydroxy-progesterone (ethinyl testosterone). *J. Endocrinol.* 1: 332. 1939.

Anhydro-hydroxy-progesterone has been found to possess progesterone-like, metrotrophic, androgenic and estrogenic properties. It is the only substance so far described which has all these properties. In the capon growth test it was found to have an androgenic activity equal to 1/600 that of injected testosterone. It was equally effective by injection or by mouth. It has 1/10 of the activity of injected progesterone in producing progestational proliferation in rabbits, either by mouth or by injection. It produces growth of the uterus without previous treatment with estrogen and it causes cornification of the vagina of the spayed rat or mouse and vaginal opening in the spayed rat. It is more active in these last two directions by injection than orally.—G. B.

FALCO, A. J. Treatment of cryptorchidism with testosterone propionate (Tratamiento de la criptorquidia por medio del propionato de testosterona). *Semana méd.* 46: 1342. 1939.

Five cases of unilateral cryptorchidism in boys from 8 to 11 years of age were treated with 5 mg. testosterone propionate, given twice weekly. Four showed complete descent within 2 to 3 months. Two cases of bilateral cryptorchidism, aged 6 and 8 years, were given doses from 5 to 10 mg. The older child gave complete response, the younger only partial, but the general conditions were quite improved, especially asthmatic attacks were attenuated and became rarer. Emphasis was laid upon the necessity of treatment in early childhood. —A. E. M.

FREED, S. C., O. HECHTER AND S. SOSKIN. Inadequacy of the vaginal smear in the rat as an index of ovarian dysfunction caused by diet. *J. Endocrinol.* 1: 268. 1939.

Rats, under certain dietary conditions (i.e., a low protein diet, a diet containing 4% of NH_4Cl, and a diet containing 8% $NaHCO_3$) were found to develop vaginal estrus in the presence of uterine diestrus and ovarian atrophy. The authors suggest that the ovary secretes one or more estrogenic factors which are all required for a complete estrus reaction.—G. B.

FRIEDGOOD, H. B., AND H. L. WHIDDEN. The assay of crystalline and urinary androgens with special reference to their measurement by a colorimetric method. *New England J. M.* 220: 736. 1939.

The Oesting colorimeter for androgenic substances was calibrated against crystalline androsterone using a modification of the Zimmerman reaction. The amount of color produced was increased by heating the reagents in a more concentrated form and then diluting to volume. Between 2.0 u and 5.0 u on the color disk each Oesting u was found to be equivalent to 8.96 to 10.00 γ crystalline androsterone. The androgen excretion for normal young women varied from 4 to 15 mg. of androsterone; that for patients with benign hirsutism and adrenogenital virilism was 1 to 29 mg. and in cases of adrenocortical tumor the value was 45 to 325 mg. In 1 case the androgen excretion fell to normal levels after removal of the tumor, but began to increase again before clinical evidence of recurrence was detected.—J. M. L.

HACHMEISTER, W. Fetal death and organ changes induced by a synthetic estrogenic material (4, 4'-dioxy alpha beta diethylstilbene) (Fruchttod und Organveränderungen durch eine östrogen wirksame synthetische Substanz). *Zentralbl. f. Gynäk.* 63: 2657. 1939.

To test out the toxicity of stilbestrol 15 guinea pigs were injected daily with 1 mg. of stilbestrol for 5, 10, or 15 days during the first period of pregnancy. The fetal deaths, maceration of tissues, and changes in liver, adrenals, and ovaries are detailed, and are more marked with the longer periods of treatment. Abortions are not reported. —E. L. S.

HARSH, R., M. D. OVERHOLSER AND L. J. WELLS. Effects of oestrogen and androgen injections on reproductive organs in male rats and mice. *J. Endocrinol.* 1: 261. 1939.

Metaplasia was induced in the epithelium of various reproductive organs of ♂ rats and mice by the injection of estrogens. In mice the estrogen administration resulted also in penis extrusion, dilatation of bladder and thickening of the submucosa of intraprostatic part of the vasa deferentia. These pathological changes were greater in castrated than in unoperated animals. Administration of testosterone was effective in preventing metaplasia of the anterior prostate glands.—G. B.

KERVOKIAN, A. Y. The treatment of chronic pruritus vulvae with local applications of estrogen. *New England J. M.* 220: 661. 1939.

In 4 cases of leukoplaki vulvitis the application of estradiol in sesame oil or incorporated in a lanolin base to the affected areas caused marked relief of the symptoms. The usual dosage was 60,000 I.U. for 2 to 6 days.—J. M. L.

KONRAD, E. A new use for inunctions of testosterone propionate (Eine neue Anwendungsart des einreibbaren Testosteronpropionates (Anertanöl Boehringer)). *Zentralbl. f. Gynäk.* 63: 2429. 1939.

Doses of about 5 γ are rubbed in 3 times daily for not over 5 days to prevent lactation in 16 women after delivery. Results were very satisfactory.—E. L. S.

NOBLE, R. L. Effects of continuous oral administration of aqueous diethylstilboestrol solutions to rats. *J. Endocrinol.* 1: 128. 1939.

An aqueous solution of diethylstilbestrol was administered to rats by using it to replace their drinking water. A daily dose of 2 to 3γ of stilbestrol caused a slight but definite inhibition of body growth in ♂ animals and a reduction of lactation in ♀ animals. A daily dose of 7 γ caused atrophy of the gonads. Larger doses of stilbestrol resulted in enlargement of the adrenals and pituitary glands, atrophy of gonads, and extreme alterations in body growth and lactation. Body growth of young rats was only slightly retarded. Anterior pituitary extracts were not able to restore lactation to normal in rats receiving oral stilbestrol, but lactation returned to normal when the stilbestrol administration was discontinued. Rats on oral stilbestrol administration reduced their fluid intake. This did not occur in immature rats but it was present in hypophysectomized rats also.—G. B.

NOBLE, R. L. Direct gynaecogenic and indirect estrogenic action of testosterone propionate in female rats. *J. Endocrinol.* 1: 184. 1939.

Stimulation of mammary glands was found in ovariectomized adult ♀ rats following the daily injection of 2 mg. of testosterone propionate for 3 to 4 weeks. The vaginal smears during this period were diestrus and there was mucification of the vaginal epithelium. In hypophysectomized animals treated in the same way there was no development of mammary tissue, the vaginal smears were estrus and the vaginal epithelium was stratified or cornified. A vaginal estrus was obtained when injections were not commenced until 3 weeks after removal of the pituitary gland. In normal immature ♀ rats, testosterone as a single dose or injected daily, caused stimulation of ovarian follicles. In hypophysectomised immature rats testosterone propionate had no effect on the ovary and produced typical vaginal mucification. Stratification of the vaginal mucosa was produced by testosterone propionate in immature hypophysectomised rats which had received preliminary ovarian stimulation by PMS extract. In all immature rats treated with testosterone propionate vaginal opening occurred, and in all injected animals there was stimulation of nipples, preputial glands, ♀ prostate and uterus. Progesterone was without effect in hypophysectomized mature or immature rats. In intact immature rats progesterone caused a decrease in ovarian weight.—G. B.

NOBLE, R. L. Effects of synthetic estrogens and carcinogens when administered to rats by subcutaneous implantation of crystals or tablets. *J. Endocrinol.* 1: 216. 1939.

As a result of the subcutaneous implantation of crystals or tablets of a number of synthetic estrogens and carcinogens into rats, it was found that only those crystals possessing estrogenic activity had an observable effect. In the adult animal there was atrophy of the gonads and reduction or cessation of body growth. There was an increase in weight of the adrenals and pituitary glands. In immature animals the gonads remained in an infantile state, body growth was retarded only slightly at first and later the animals grew more slowly. In ♀ rats, after 3 weeks' treatment with estrogen crystals, extracts of PU caused an ovarian response similar to that obtained from the normal immature rat. After tablet implantation of 18 weeks, PU produced an effect on the ovaries similar to that observed in hypophysectomized rats.—G. B.

OGSTON, A. G., J. ST. L. PHILPOT AND S. ZUCKERMAN. Observations relating to the swelling of sexual skin in Rhesus monkeys. *J. Endocrinol.* 1: 231. 1939.

The presence of a mucoprotein-like substance in the viscous exudate from swollen sexual skin of monkeys is recorded. Examination of this exudate in the ultracentrifuge showed 3 main sedimentation boundaries. Two resembled normal proteins and the third which was abnormally sharp, with a sedimentation constant of 2.7–3.7 $\times 10^{-13}$ was believed to represent the mucoprotein-like substance. Exudate from chopped cock's comb and chopped human umbilical cord also gave an abnormally sharp sedimentation boundary. The physical properties of the mucoprotein-like substance suggest that it is a mucoprotein but the chemical evidence is inconclusive. In view of the low concentration of the substance in the exudate it is thought unlikely that it contributes appreciably to the swelling of the sexual skin by water. There is some evidence that the high chloride concentration which is present in the exudate may play a part in the mechanism of sexual skin swelling.—G. B.

REYNOLDS, S. R. M., AND F. J. FOSTER. Peripheral vascular action of estrogen observed in the ear of the rabbit. *J. Pharmacol. & Exper. Therap.* 68: 173. 1940.

Intramuscular injections of estrone, estradiol, estradiol-dipropionate and amniotin in 13 ovariectomized rabbits produced dilatation of the minute blood vessels of the ear. The effect commences within a few minutes and reaches a maximum in 6 to 20 minutes. The ground color of the ear changes from pale to pink. Temperature changes in the skin of the ear, measured by a thermocouple-potentiometer system, were induced by these drugs.—E. L.

SELYE, H. Morphological changes in female mice receiving large doses of testosterone. *J. Endocrinol.* 1: 208. 1939.

Adult ♀ mice showed a decrease in size of ovaries, disappearance of normal corpora lutea and marked follicle stimulation with development of occasional follicular and corpus luteum cysts following 20 daily injections of 5 mg. of testosterone propionate. The adrenal cortex became atrophic and the hypophysis decreased in weight. In the kidneys there was present hypertrophy of proximal and distal convoluted tubules and the parietal lamina of Bowman's capsules. There were no changes in liver and spleen. There was no evidence of toxic action resulting from such high doses of testosterone propionate.—G. B.

SIEGLER, S. L., AND L. M. SILVERSTEIN. The use of testosterone propionate in the inhibition of lactation during the puerperium. *Am. J. Obst. & Gynec.* 39: 109. 1940.

Success was obtained in 47 of the 50 cases treated. The doses were 10 to 25 mg., given 3 to 5

times, the total varying from 30 to 125 mg. In 33 cases the total was only 30 mg. Treatment began from the second to the seventh day, usually on the third day. There were no afterpains or alteration in post-partum bleeding attributable to this therapy.—E. L. S.

STROUD, S. W. Recovery of injected estrogenic substances from rabbit urine. *J. Endocrinol.* 1: 201. 1939.

The author describes a technique for the extraction of synthetic estrogens from the rabbit's urine. The results of recovery of synthetic estrogens from rabbit's urine were compared with that of estrone under the same conditions. A non-ketonic phenolic metabolic product of estrone which was probably β estradiol, was found in the urine of rabbits receiving large injections of estrone.—G. B.

STURGIS, S. H. The diagnostic significance of the endometrial glands in early pregnancy. *Am. J. Obst. & Gynec.* 39: 10. 1940.

From a series of some 1500 endometrial biopsies 11 are reported because of changes from the usual results of progesterone in the late 3rd and early 4th weeks of the normal menstrual cycle. The epithelial cells are larger in all dimensions, bulging into the gland lumina, with well marked granulations save at the free margin where there is a fine reticulum and a distinct cell membrane. The nuclei are basal, and no mitoses are seen. Ten were proved pregnant, and the other was probably pregnant. This may prove to be a very early diagnostic sign of pregnancy, especially useful in cases of ectopic pregnancy, of which there were 2 in this series.—E. L. S.

STURGIS, W. C., A. R. ABARBANEL AND D. S. NADER. Treatment of metromenorrhagia with testosserone propionate. *Am. J. Obst. & Gynec.* 39: 102. 1940.

The rationale of testosterone therapy for uterine bleeding is that this hormone reduces contractility of the myometrium, and lessens the blood flow to and in the uterus. The minimal doses to lessen bleeding were 10 to 30 mg. The total dose to stop flows was 10 to 120 mg., with 40 to 60 mg. commonest. Two to four doses were given at 2 to 4 day intervals. Subcutaneous injection was more effective, intramuscular secured more rapid initial action. Results are reported in 14 cases, ages 15 to 48.—E. L. S.

TSCHERNE, E., AND W. P. SCHAEFFER Precocious puberty associated with ovarian chorionepithelioma (Pubertas praecox durch ein Chorionepitheliom des Ovarium). *Zentralbl. f. Gynäk.* 63: 2417. 1939.

A case of precocious puberty in an 8-year old girl was found to depend on chorionepithelioma of 1 ovary, as a result of which the other ovary contained follicular cysts, and the uterus and tubes were enlarged to adult size. The urine gave a reaction in rabbits simulating that from pregnant women.—E. L. S.

ZUCKERMAN, S. The effect of sex hormones, cortin and vasopressin on water retention in the reproductive organs of the monkeys. *J. Endocrinol.* 1: 147. 1939.

Daily increasing doses of estrone (100γ to 400γ) injected intramuscularly into a normal pig-tailed monkey during the post ovulation stage of the menstrual cycle led to swelling of the sexual skin due to accumulation of water. The absence of sexual skin swelling during this phase in the normal cycle shows that the progesterone formed by the corpus luteum is not able to retain the sexual skin water which accumulates as a result of pre-ovulation estrogenic stimulation. Testosterone propionate and cortin were not able to cause retention of sexual skin water which had been deposited as a result of previous injections of estrone. Vasopressin however did cause retention if it was injected at short intervals. Progesterone and testosterone inhibited post estrogen bleeding in monkeys, cortin and vasopressin did not—G. B.

HYPOPHYSIS

CROOKE, A. C., AND R. K. CALLOW. The differential diagnosis of forms of basophilism (Cushing's syndrome), particularly by the estimation of urinary androgens. *Quart. J. Med.* 8: 283. 1939.

Four cases presenting the clinical syndrome of basophilism were described. Two were proven to have suprarenal tumors, 1 had a basophile adenoma of the pituitary without adrenal neoplasm or hyperplasia; in the 4th exploration revealed no suprarenal tumor but the status of other glands is not known. Androgen determinations were made on the urine of all these patients both by colorimetric assay of 17-ketosteroids and by biological assays with the capon comb method. The urines of the 2 patients with suprarenal tumors showed high androgen values with both tests. This increase was found due largely to transdehydroandrosterone. Of the other 2 cases, without suprarenal tumors, 1 showed approximately normal levels of androgen excretion; whereas the other, tested only by biological method, showed levels below normal. After surgical removal of the suprarenal tumor in the 25-year old ♂ (patient 1) the steroid assay in the urine fell well below normal but had returned to normal levels 6 months later. The authors felt that urinary assay of 17-ketosteroids gives valuable assistance in determining the presence of suprarenal cortical tumors in basophilism.—J. E. H.

EHRHARDT, K., AND H. F. VOLLER. Lactogenic hormone of anterior pituitary (Untersuchungen über das Laktationshormon des Hypophysenvorderlappens). *Endokrinologie* 22: 19. 1939.

Studies included the determination of lactogenic hormone in human blood and urine on different days of the menstrual cycle, and the treatment of guinea pigs by inunction or intraperitoneal injection of a commercial product. It was found that the amount of lactogenic hormone in the blood and urine shows two peaks, one at the beginning of menstruation and a second at the time of ovulation. Menstrual blood was found to contain large amounts of hormone. Adm. of the hormone to pregnant guinea pigs caused abortion. The authors conclude that the lactogenic hormone of the anterior pituitary acts not alone on mammary tissue but also in part on the ovary and on the endometrium.—W. J. A.

GAEBLER, O. H. AND P. BARTLETT. Effects of anterior pituitary preparations and iodine on nitrogen excretion, creatinuria and basal metabolism. *J. Biol. Chem.* 129: 559. 1939.

The nitrogen and creatine output of unoperated and thyroidectomized bitches and the B.M.R. were determined after giving iodine, thyrotropic hormone and growth hormone. Thyrotropic hormone caused a marked increase in the creatine output but had no effect on weight or nitrogen output. Growth hormone caused an increase in the B.M.R. and body weight but a decrease in nitrogen, phosphate and creatine. Desiccated anterior pituitary powder by mouth was entirely without effect. Lugol's solution had no effect on the excretion of nitrogen creatine or creatinine and the thyrotropic hormone, likewise, produced no change in thyroidectomized animals.—J. M. L.

KERPPOLA, W. Concerning the symptoms of hypophyseal dysfunction in cases of underweight (Über Symptome von Hypophysenschwäche in Fällen von Untergewicht). *Acta med. Scandinav.* 99: 287. 1939.

Forty patients whose chief complaint was underweight with no essential dietary basis were studied for symptoms of hypophyseal dysfunction. More than half showed a lowering of blood sugar values during the sugar tolerance test, a relative lymphocytosis and a lowered blood chloride level. In about ⅓ of the cases the sella turcica was under normal size and there was a slight anemia and a granulocytopenia. Menstrual difficulties, lack of growth and a low B.M.R. were found in a few cases. The severity of the symptoms bears no direct relationship to the amount of underweight and no definite symptom complex was found in most cases.—J. M. L.

KÖHNE, G. Diabetes mellitus and the hypophysis-midbrain system (Diabetes mellitus und Hypophyse-Zwischenhirnsystem). *Endokrinologie* 22: 241. 1939.

From studies which include clinical-pathological observations on 10 human cases the author is led to conclude that classical diabetes is not to be ascribed to primary changes in the pituitary or the mid-brain. On the other hand certain of his cases show that changes in this region may influence diabetes and alter its clinical course in an atypical manner.—W. J. A.

KYLIN, E., AND E. DICKER. The rôle of the hypophysis in the pathogenesis of total alopecia (Le rôle de l'hypophyse dans la pathogénie de l'alopécie totale). *Acta med. Scandinav.* 100: 485. 1939.

The authors studied 29 patients with total alopecia. They found no marked disturbance in nutrition, B.M.R. or blood chemistry. The loss of hair was complete over the entire body and is found in all ages and sexes. The affliction was hereditary and obeyed the Mendelian laws. The condition was due to disturbance of the endocrine glands, chiefly the hypophysis. Implants of total hypophyseal glands from calves gave as encouraging results as injections of extracts of the gland. —J. M. L.

SEVERINGHAUS, A. E., AND K. W. THOMPSON. Cytological changes induced in the hypophysis by the prolonged administration of pituitary extract. *Am. J. Path.* 15: 391. 1939.

Cytological studies were made on the anterior lobe of the hypophyses from: (a) dogs injected for extended periods with sheep pituitary extract; (b) dogs injected with canine antihormones, and (c) ewes after prolonged injection of sheep pituitary extract. Marked changes were observed in the hypophyses studied and in the dogs there was evidence of atrophy of the thyroid gland, the gonads and possibly of the adrenals. In interpretation, the authors assumed the following reactions to occur in sequence in the experimental animals: 1. Injection of pituitary extract activates the endocrine glands related to the anterior lobe, namely gonads, thyroid, and probably adrenals. 2. The increased secretion of these activated glands in turn stimulates the anterior lobe which augments with its own secretion the injected extracts. 3. The excess of extract from a foreign species results in the production of antihormones. 4. The antihormones at first neutralize the injected pituitary extract, and later the secretion of the hypophysis of the injected animal itself. Thereafter the end organs (gonads, thyroids, adrenals) react by atrophy as in states of pituitary insufficiency, even though the hypophysis continues

its activity. 5. The atrophy of these related glands in turn brings about in the anterior lobe certain changes which are characteristic of gonadectomy and thyroidectomy.—W. J. A.

SHARPEY-SCHAFER, E. P., AND I. SCHRIRE. The effect of pituitary thyrotropic extract in subjects with low basal metabolic rates. *Quart. J. Med.* 8: 195. 1939.

Various types of patients were given intramuscularly 100 to 300 Heyl-Laqueur units of thyrotropic principle per day for 3 to 6 days. Observations of pulse, temperature, size of thyroid gland, etc., were made, as well as urinary creatine studies and basal metabolic rates. Of 22 normal subjects all gave prompt response to thyrotropic principle, generally within 3 days, as judged by all the above methods of study. Two normal subjects were given 3 times larger doses with little if any greater response. Three subjects with myxedema and 1 cretin failed to show any response whatever. Five subjects with low B.M.R. but with no clinical evidence of hypothyroidism responded as did the normals to the injections. Three subjects with low B.M.R. but with no clinical evidence of hypothyroidism failed to respond even to large doses of the extract. The authors concluded that thyrotropic extracts may be used to investigate the function of the thyroid gland in man, and that its further use may help clarify that indefinite group of patients with low B.M.R. but without other signs of thyroid deficiency.—J. E. H.

SHEEHAN, H. L. Simmonds's disease due to postpartum necrosis of the anterior pituitary. *Quart. J. Med.* 8: 277. 1939.

Most cases are believed due to late effects of postpartum necrosis due to hemorrhage or collapse at time of delivery. Detailed analyses of 51 cases revealed 32 in which the clinical diagnosis was confirmed at postmortem, as indicated by marked reduction in size of the anterior lobe, with predominance of chromophobes, a small thyroid with histological evidence of reduced function. Suprarenal cortices showed markedly atrophy, dissimilar to that seen in Addison's disease. Ovarian atrophy and diminution in estrogenic hormones was reflected by involutional changes in the genitalia. Other endocrine glands showed no change. The clinical picture varied greatly depending on the degree of pathological change in the glands indicated, and perhaps in the number and types of residual cells in the anterior pituitary. Blood loss or puerperal sepsis early in puerperium usually evoked a clinical condition which obscured signs of pituitary insufficiency. Suggestive evidence of Simmonds's disease soon after delivery was indicated by failure of lactation and by hypoglycemia. Little evidence was found to support the view that emaciation is a characteristic of the syndrome; actually most of the authenticated cases were either normal or above normal in weight, and reduced food intake is felt to explain emaciated cases. The B.M.R. was always low, and some degree of hypochromic anemia was present. Many spurious cases have been reported in recent literature, and the general picture of the syndrome thus distorted.—J. E. H.

BAATZ, H. The effect of prolan on spontaneous tumor growth in mice (Die Beeinflussung des Spontantumorwachstums der Maus durch Prolan). *Ztschr. f. Geburtsh. u. Gynäk.* 120: 70. 1939.

Injections of prolan in oil every 3 to 4 days over a period of 10 days into 54 ♀ mice with 58 spontaneous tumors resulted in no inhibition of tumor growth, increased growth in 1/3 of the animals and no effect in 2/3 of them. The effect could not be correlated with the amount injected. The total dose varied from 100–200 R.U. The growth of the tumors was measured for 10 days before the injections, 10 days during and 10 days after the injections were ended.—M. R. WHITE

CHANCE, M. R. A., I. W. ROWLANDS AND F. G. YOUNG. Species variation in thyrotropic, gonadotrophic, and prolactin activities of anterior hypophyseal tissue. *J. Endocrinol.* 1: 239. 1939.

Acetone desiccated anterior pituitary glands of pig, man and sheep contain greater amounts of thyrotropin, gonadotropin, and prolactin than those of horse or ox. The pituitaries of all 5 species of animal showed substantial gonadotropic activity when assayed by ability to induce ovulation in the estrus rabbit. When assayed by capacity to cause ovarian growth in the immature rat, however, man horse and ox pituitaries were found to possess only a slight activity. The thyrotropic extracts were prepared by the Van Dyke acid-buffer method and by the Bates-Riddle alkaline—aqueous—alcohol method. Both were satisfactory. The Bates-Riddle method was also used for the preparation of pro lactin. Van Dykes acid buffer method was used foı the preparation of gonadotropic extracts.—G. B.

DANDY, W. E. Section of the human hypophysial stalk: its relation to diabetes insipidus and hypophysial functions. *J.A.M.A.* 114: 312. 1940.

Division of the stalk without trauma to adjacent structures resulted in a permanent polyuria and polydipsia which still persists after 11 years. There resulted no obvious disturbance of known hypophysial functions, as reflected in menstruation, pregnancy, lactation, weight changes and blood pressure. Sugar content of urine has remained normal. In this case therefore division of the stalk was solely responsible for the subsequent high level of

water exchange, and it is believed the hypophysis itself is not concerned.—H. O. H.

DEANESLY, RUTH. Modification of the effectiveness of gonadotrophic extracts. *J. Endocrinol.* 1: 307. 1939.

A review of the work on this subject and a record of tests carried out over several years on the modification of gonadotropic effectiveness.—G. B.

FISCHER, S. Treatment of schizophrenia with glandular extracts. *Arch. Neurol. & Psychiat.* 42: 644. 1939.

The author has previously published considerable evidence that the schizophrenic psychosis is characterized by a depression in the specific dynamic reaction to ingested protein. From this finding the deduction is drawn that pituitary deficiency is an important etiological factor in the psychosis. In a therapeutic test of the hypothesis a PU extract, "praehormone" was administered to 96 patients. Of 50 who had been psychotic less than 6 months 42 showed complete remission and 8 improvement. In the 43 whose illness was of longer duration the remissions were 17 and improvements 14. The material was given in 15 daily injections with repetition of the course, if necessary, after a month's interval—R. G. H.

GARDINER, S., AND J. T. BRADBURY. Responses of the human post-partum uterus to posterior pituitary extracts. *Am. J. Obst. & Gynec.* 39: 1. 1940.

By kymographic records with a bag in the human uterus during the puerperium the authors studied the responses to pituitrin, pitressin, and pitocin. Doses of 0.3 cc. of these 3 provoked similar responses. In spite of wide variation in assayed value, dilutions of pitocin and pitressin produced similar effects, when dilutions were equivalent, but not when diluted parallel to their oxytocic assays. Therefore the commercial assays do not give a measure of the oxytocic activity of pitressin. There was no evidence of a tolerance nor of inhibition in the human uterus, although in a series of tests the later trials give a decreased response. The authors suggest a species difference as explanatory for the effects noted. The report is based on data from 60 patients.—E. L. S.

GUERCIO, F., AND D. CAZZOLA. The antogonadotropic quality of the serum of dogs treated with pituitary-like gonadotropic hormone (Proprietà antigonadotropa del siero di cane trattato con gonadotropo simile ipofisario). *Arch. di fisiol.* 39: 372. 1939.

Treatment with prolan produced antihormones in the dog and the rabbit. The potency of the serum could be maintained for an indefinite time by maintaining adequate treatment. Positive complement deviation and precipitin tests were obtained in the rabbit but not in the dog. Quantitative relationships between serological tests and antihormone potency did not exist. Simultaneous application of prolan and antihormone serum left ovaries of tests animals unaffected.—A. E. M.

GUERCIO, F., AND D. CAZZOLA. The effect of ovariectomy and splenectomy on the antigonadotropic characteristics of rabbit serum (Effetti dello smilzamento e della ovarectomia sulle caratteristiche dei sieri antigonadotropi del coniglio). *Arch. di fisiol.* 39: 396. 1939.

Splenectomized rabbits did not develop antibodies against urinary gonadotropic hormone, and splenectomy after treatment caused disappearance of the antibodies from the blood. The antihormonic effect, however, was not influenced by removal of the spleen. The identity of antihormones and antibodies is therefore denied. Ovariectomy did not prevent the formation of antigonadotropic hormones.—A. E. M.

GUERCIO, F., AND D. CAZZOLA. The relationship between antihormonic serum and zoölogical specificity (Osservazioni sui rapporti tra siero antihormone e specificita zoologica). *Arch. di fisiol.* 39: 407. 1939.

Three times the minimal dose of PU sufficient to produce a positive reaction in the virgin rabbit was ineffective if 1 hour previously 10 cc. of blood had been injected taken from a dog that had received treatment with urinary gonadotropic substance.—A. E. M.

KYLIN, E. Hypophyseal transplantation in a case of diabetes insipidus (Hypophysentransplantation in einem Fall mit Diabetes insipidus). *Acta med. Scandinav.* 101: 566. 1939.

A girl of 22 who developed diabetes insipidus was treated by injection of finely divided hypophyseal gland into the gluteus. The polyuria decreased from a maximum of 5800 ml. daily to 800 ml. and the sp. gr. increased from 1.001 to 1.020. The polydipsia ceased and the patient felt completely well.—J. M. L.

LEWIS, A. A., AND C. W. TURNER. The mammogenic hormones of the anterior pituitary. The duct-growth factor. *Univ. Missouri Col. Agr. Exp. Sta. Bull.* 310. 1939.

Whereas ovarian hormone action on growth of duct and lobule-alveolar systems in indirect, the anterior pituitary secretes two mammogenic factors which directly activate glandular growth. One factor stimulates the duct system, and one the lobule-alveolar system. The latter factor is considered in the present report, its presence and

activity being based upon an assay method using the male mouse. A low level was found in glands of fetuses, growing males, and steers. It was low in beef and dairy pituitaries in early and late pregnancy, rising to a peak at about the 150th day. Dairy-cow glands exceeded considerably those of beef cows in mammogen assay. And even higher content was found in growing heifers than in pregnant females, while lactating cows had more than dry cows. In the oestrous cycle, it appeared highest in the luteal phase. Male rabbits given estrone responded with an increase in mammogen to double the content in pregnant rabbits. With excessive estrone dosage no mammogen response was obtained. The duct-growth mammogen was readily separable from other known anterior pituitary factors. Any estrogen present in the pituitaries proved negligible in causing mouse mammary proliferation. Concentration to 1/400 of the fresh condition was obtained. It is believed that separation of a duct-growth and a lobule-alveolar factor was attained. Pituitary extracts were shown to develop complete mammary-duct systems in male and female mice, spayed female rabbits and hypophysectomized ground squirrels.—*Author's Summary, abbreviated.*

Perry, Isabella H., and Margit S. Lochhead. Experimental adenoma of the pituitary. *Am. J. Cancer.* 35: 422. 1939.

Three adenomas of the pituitary occurred in 131 ♀ mice chronically treated with estrone. No pituitary adenomas were observed in 97 controls not receiving estrone. Two of the mice with pituitary adenomas had subcutaneous implants of 1.2.3.6 dibenzanthracene in addition to the estrone treatment. The third case had no other treatment. It also had a mammary carcinoma. Hyperplasia, hyperemia and necrosis occurred in the pituitaries of estrone-treated mice. The literature of pituitary adenomas in rodents is briefly summarized.—*Authors's Abstract.*

Preissecker, H. Present status of endocrine therapy for hypogalactia (Der gegenwärtige Stand einer Hormonbehandlung der Hypogalaktie). *Zentralbl. f. Gynäk.* 63: 2539. 1939.

After reviewing the physiology of lactation and the disappointing results of therapy with even recent prolactin preparations, the author refers to his experience with inunction of a mixture containing 100 pigeon u of prolactin per 20 gm. Ten times this concentration is not obviously better. Secondary failure of lactation is said to respond better than primary cases. Rectal administration of 300 u daily has also been found helpful. The author emphasized the importance of development of the breasts during gestation. (No protocols.)—E. L. S.

Rakoff, A. E. The hormonal diagnosis of intra-uterine fetal death. The value of quantitative serum prolan determinations as a diagnostic procedure. *Pennsylvania M. J.* 43: 669. 1940.

In intra-uterine fetal death, quantitative determinations of the serum prolan show a progressive fall in the concentration of this hormone. Although there is also a fall in serum estrogen, this is not technically as easy to determine, nor are the values as significant, especially in the first half of gestation. The serum prolan values may fall below the normal concentration for a number of days or weeks before it becomes sufficiently low for the Friedman pregnancy test to become negative. Fourteen patients were studied in whom death of the fetus occurred but in whom the Friedman test on admission was positive. In all but 2 instances the serum prolan values were below normal, and in 10 cases they were less than 100 M.U. per 100 c.c. Serum prolan values of less than 100 M.U. per 100 c.c. are indicative of intra-uterine fetal death, despite the presence of a positive Friedman test. When the serum prolan falls to 50 M.U. or lower, the Friedman test is usually but not always negative. Quantitative determinations of the serum prolan at the 50 and 100 M.U. level yield information of greater value than the Friedman test in patients with suspected or threatened death of the fetus. The procedure is simple and inexpensive. Complete quantitative estimations of the serum gonadotropic hormone, especially when repeated at intervals, frequently yield information of prognostic value.—I. B.

Randall, L. M., T. B. Magath and F. N. Pansch. A study of "false" Friedman tests. *J.A.M.A.* 114: 471. 1940.

Of 645 Friedman tests 2.63% were at variance with the ultimate diagnosis. Inaccuracies occur if urinary dosage is not kept within the original Friedman limits of 8 to 15 cc. Larger dosages may increase positive responses, but diagnoses decrease in accuracy. Similarly, a positive response with smaller amounts of test urine may indicate a greater APL content than is normally associated with pregnancy. So-called false positives can then be eliminated by injection of small amounts of urine. The Friedman test is therefore quantitative. Excretion of gonadotropic principle in excessive amounts may elicit a positive Friedman reaction in the absence of pregnancy. Such a circumstance may arise pathologically through hydatidiform mole or chorio-epithelioma, and may occur physiologically in the menopause, in menstrual disorders such as primary ovarian failure, and in treatment with anterior pituitary preparations, and, finally, through errors in technic. A test giving negative results before the 7th week should be repeated later. The fact is emphasized that the test, biologically, is

based on the presence of gonadotropic substance in urine, and that, only with proper precautions, is it a test of pregnancy.—H. O. H.

REPORT OF THE COUNCIL ON PHARMACY AND CHEMISTRY. The present status of therapy with chorionic gonadotropin. *J.A.M.A.* 114: 487. 1940.

This report embraces a summary of the clinical experiences with chorionic gonadotropin and an evaluation of results. No satisfactory rationale exists for treatment of uterine bleeding with this substance. Reports have been conflicting and the general concensus is one of skepticism of its value. Moreover, the possibility of ovarian damage, in view of its action in the primate, is not to be overlooked, although from available evidence such damage is perhaps not of permanent nature. Results of treatment of cryptorchidism have been more promising, although the possibility of spontaneous descent must be recognized. Analysis of results has revealed that cures are by no means as frequent as reported in earlier literature, that most cases which have responded would show descent spontaneously at puberty. However, cases not responding require surgery, and surgery at an early age is desirable. It has also been claimed that endocrine therapy aids in surgical treatment of undescended testicles, both pre- and postoperatively. In view of these observations therefore, therapy may be of value in cryptorchidism, if its limitations be recognized. No final concensus may be established as yet with respect to its use in hypogonadism. Its use in stimulation of spermatogenesis has been disappointing. Its use in a variety of conditions such as enuresis, migraine, peripheral vascular disease has failed to produce results which support therapeutic claims. In summary, its use for cryptorchidism appears the only one with a substantial basis.—H. O. H.

RINDERKNECHT, H., AND P. C. WILLIAMS. Separation of follicle stimulating fraction from pituitary gonadotrophin. *J. Endocrinol.* 1: 117. 1939.

The authors were unable to separate a pure follicle stimulating fraction from horse pituitary extracts by the methods Evans et al., and of Fevold. Modifications of these methods resulted in a relatively pure fraction being obtained. It is suggested that certain inert materials present in crude extracts seriously interfere with the separation of LH and FSH since removal of these inert materials from the crude extracts improved the results.—G. B.

ROWLANDS, I. W. The rate of appearance of anti-luteinising activity in the serum of rabbits injected with extract of ox pituitary glands. *J. Endocrinol.* 1: 172. 1939.

There is a selective inhibition in immature rats of the luteinising activity of a gonadotropic extract of gelding pituitary gland by the serum of rabbits injected daily with a gonadotropic extract of ox pituitary gland. The amount of anti-luteinising activity present in the serum increases up to about 7 weeks after the commencement of immunisation, is constant during the next two weeks, then falls somewhat.—G. B.

ROWLANDS, I. W. Further observations on the progonadotrophic and antithyrotrophic activity of antisera to extracts of the anterior pituitary gland. *J. Endocrinol.* 1: 177. 1939.

The gonadotropic activity of ox and pig pituitary gland was augmented by the serum of a dog injected daily for many weeks with an extract of ox pituitary gland. The serum had no effect on the thyrotropic activity of the extract used for immunisation. The effect of an extract of pig pituitary gland on immature mouse ovaries was augmented by the serum of a goat injected with this extract.—G. B.

STEPHEN, D. J. Chloride excretion in hypopituitarism with reference to adrenocortical function. *Am. J. M. Sc.* 199: 67. 1940.

A girl of 15 who had suffered most of her life from traumatic epilepsy was subjected to therapeutic cauterization of her hypophysis. The procedure was followed by temporary disturbance of water and salt metabolism and of temperature regulation. Later loss of weight, decrease of B.M.R., hypotension (70 mm. Hg.) disappearance of pubic and axillary hair, amenorrhea and regression of the genitalia occurred. A second patient had the signs of generalized infantilism. A cystic adenoma of the pituitary was found and removed. Five other cases are described in which the diagnosis of hypopituitarism was convincingly made on clinical grounds—especially depression in the sex sphere. Six of the 7 patients, when studied under standard conditions of salt depletion, showed increased concentrations of chloride in the urine similar to that characteristic of adrenocortical insufficiency. Four of the 6 patients developed symptoms suggesting Addisonian crisis. These symptoms were promptly relieved by the intravenous administration of sodium chloride, glucose and adrenal cortex extract. In 2 patients symptoms failed to occur and chloride excretion was favorably modified after administration of sodium chloride and adrenal cortex extract. One patient experienced striking clinical improvement during the administration of sodium chloride and adrenal cortex extract. These observations are interpreted as confirmatory evidence of the occurrence of chronic adrenocortical insufficiency in clinical hypopituitarism, presumably secondary to withdrawal of the adrenotropic anterior pituitary principle.—R. G. H.

VAN DEN ENDE, M. Precipitins in antigonadotrophic sera. *J. Endocrinol.* 1: 156. 1939.

Precipitins were demonstrated in antisera to active gonadotropic extracts of PU. The presence of precipitin was independent of the hormone activity of the extract. No constant relationship was found between precipitin content and antigonadotropic power. Extracts of PU were found to contain multiple antigens.—G. B.

VAN DEN ENDE, M. Urinary gonadotrophic extracts and anaphylaxis in vitro. *J. Endocrinol.* 1: 356. 1939.

Confirmation of the presence of antibodies by precipitation tests on anti-gonadotropic serum has been obtained by anaphylactic sensitisation. The isolated uteri of guinea pigs react specifically to contact with urinary extracts irrespective of their hormone contents. Sensitivity to human serum is also induced but anaphylactic reaction to urinary extract is present even after specific desensitisation to serum. By active sensitisation to urinary extracts, a higher degree of sensitivity to species (serum) antigen is induced than by passive sensitisation. This can be explained by the occurrence of serum proteins in the urinary extracts. The demonstrable antibodies evoked by urinary gonadotropin are not hormone specific.—G. B.

YOUNG, F. G. Anterior pituitary fractions and carbohydrate metabolism. I. The preparation and properties of diabetogenic extracts. *J. Endocrinol.* 1: 339. 1939.

Globulin, pseudoglobulin, euglobulin and albumen fractions were prepared from ox anterior pituitary by fractionation with ammonium sulphate at 0° C. Only the globulin and pseudoglobulin fractions were found diabetogenic on daily injection into normal dogs. The diabetogenic fractions possessed thyrotropic, gonadotropic, and glycotropic activities but preparations with these properties were not necessarily diabetogenic. The diabetogenic extracts were found to possess greater growth-promoting activities than non-diabetogenic fractions. The diabetogenic fractions gave positive Biuret, Xanthoproteic, Millon, and Hopkings-Cole reactions. The albumen fraction gave a positive Molisch reaction. All fractions showed the presence of sulphur. The presence of phosphorus was doubtful. Nitrogen (Kjeldahl method) varied from 13 to 16% in the 3 fractions. Dogs made temporarily diabetic by treatment with anterior lobe extracts were found to have less muscle glycogen, about the same amount of liver glycogen and more liver fat than normal dogs under the same conditions.—G. B.

PANCREAS

BAKER, A. B. Cerebral lesions in hypoglycemia. *Arch. Path.* 28: 298. 1939.

Twenty-six rabbits divided into 3 groups. Seven rabbits were subjected to severe and prolonged hypoglycemia action, allowed to live 130 days; Thirteen animals were given repeated hypoglycemic reactions at weekly intervals over a period of months, the total number of shocks varying from 7 to 19. They were allowed to live 1 to 100 days; Six rabbits were used as controls. Hypoglycemia was produced by intravenous injection of 10 to 30γ of insulin in animals starved for 18 hours. The histologic studies of the nervous system indicated widespread severe damage to the brain. Nerve cell damage however, was not so striking as the multiple cerebral hemorrhages, the demyelination, encephalomalacia and the glial reactions. No correlation could be found between total dose of insulin administered and severity of damage to the brain. It was suggested that the cerebral damage in hypoglycemia might be due to a qualitative circulatory disturbance, inasmuch as the blood reaching the brain is deficient in proper nutritive materials.—L. B. S.

BAUMAN, L. Clinical experience with globin insulin. *Am. J. M. Sc.* 198: 475. 1939.

The utility of a globin insulin and zinc compound was tested in 25 diabetic patients. The product has the advantage of reaching its therapeutic efficiency promptly and persisting about twice as long as that of plain insulin. The clinical results were satisfactory.—R. G. H.

BRAZER, J. G., AND A. C. CURTIS. Vitamin A deficiency in diabetes mellitus. *Arch. Int. Med.* 65: 90. 1940.

The occurrence of increased amounts of carotene in the blood of patients with diabetes mellitus has long been recognized both by the clinical observation of xanthosis and by the results of laboratory tests. A group of 20 patients with juvenile diabetes mellitus were studied, and all were found to have poor light adaptation by the Frober-Faybor biophotometer. Three of the group were subjectively aware of night blindness, and 9 showed cutaneous changes compatible with vitamin A deficiency. The daily administration of 60,000 U.S.P. units of vitamin A, in the form of crystalline carotene dissolved in vegetable oil, for as long as 14 days did not affect the light adaptation of the patients with diabetes mellitus. The daily administration of 60,000 U.S.P. units of vitamin A to patients with diabetes mellitus, in the form of concentrated fish liver oils, caused their light adaptation to return to normal or nearly normal in periods ranging from 3 to 21 days. The cause of poor light adaptation in pa-

tients with juvenile diabetes mellitus appears to be an inability to convert carotene to vitamin A.—I. B.

CAMPBELL, W. R., R. R. GRAHAM AND W. L. ROBINSON. Islet cell tumors of the pancreas. *Am. J. M. Sc.* 198: 445. 1939.

The authors add 5 surgically-treated cases to the approximately 100 cases of authentic islet-cell tumors reported in the literature. It was found that either α or β cells may predominate. The insulin content of the removed tissue ranged from about 2 to 85 U of insulin per gm. In 4 of the 5 cases surgical excision of the islet excess resulted in apparent cure. In addition to the well-known hypoglycemia crises, marked chronic changes in the nervous system were noted. These were variously manifested by emotional instability, mental deterioration, coarsening of the character and vulgarity in speech and deportment. Attempts to control the hypoglycemia by increased food intake resulted in some instances in obesity. Early diagnosis and surgical treatment of such cases is recommended.—R. G. H.

RICHARDSON, R. Immunity in Diabetes. III. Relation of tissue glycogen and blood chemistry to bacterial dissemination, antibody formation and survival after infection in diabetes. *J. Clin. Investigation* 19: 239. 1940.

In normal rabbits and depancreatized cats survival after intravenous inoculation with bacteria is increased by carbohydrate feedings which raise liver glycogen. A lowered nutritional state, accompanied by lowered liver glycogen occurs in depancreatized cats in which injection of B. typhosus vaccine has produced a low agglutinative titre. Alterations in blood of these animals, simulating human diabetes, does not alter survival time. Dissemination of bacteria from a skin focus in such cats seems correlated with alterations in skin glycogen, is increased by acidosis (ketosis), and is more marked in the diabetic cats than in control animals, but cannot be correlated with alterations in blood sugar, cholesterol or proteins or with liver glycogen.—E. L. S.

STROME, F. P., AND B. C. BLAINE. Diabetes. 1. Diabetes mortality in Pennsylvania. *Pennsylvania M. J.* 43: 481. 1940.

The diabetes mortality rate of the United States is exceeded by that of the Maltese Islands, but is greater than that of any other of the 32 countries for which statistics are available. Diabetes mortality rates in Pennsylvania have increased 297% from 1906 to 1937. Mortality rates from diabetes among white persons over age 40 show a tremendous increase. The most marked increase is to be found in persons almost entirely in the age groups over 45, predominantly in the age group over 65. Definite indications are found that improvement in the treatment of diabetes is lowering the death rates in ages under 40. Future death certificates should clearly specify whether a death is from diabetes mellitus or its complications, or with diabetes. For example, a diabetic dying in diabetic coma is a death from diabetes. Diabetes is "the principal cause of death." When a diabetic dies of another condition, as for instance, a death from pneumonia, the person dies with his diabetes. Diabetes should then be listed on the death certificate under "contributory" causes. A state-wide educational program for the physician and carefully written information for the laity on the prevention, diagnosis, and treatment of diabetes should be instituted.—I. B.

TALKINGTON, P. C., AND T. H. CHEAVENS. Insulin shock therapy in Korsakoff's psychosis. *J. Nerv. & Ment. Dis.* 91: 175. 1940.

A woman of 34 was admitted to hospital in delirium following prolonged abuse of alcohol. She was violent and destructive, incontinent and incoherent. Following a course of 53 insulin injections, characterized by deep "wet" shocks she recovered without residual manifestations except circumscribed amnesia.—R. G. H.

PARATHYROIDS

ANDERSON, I. A., AND A. LYALL. The treatment of chronic hypoparathyroidism. *Quart. J. Med.* 8: 209. 1939.

The authors reviewed the literature on the effect of variations in the amount of ingested phosphorus in chronic hypoparathyroidism. They presented in great detail a study of 3 cases of parathyroid insufficiency. Balance studies were made with variations in the amounts of ingested calcium and phosphorus. The conclusion was reached that in the therapy of hypoparathyroidism limitation of the phosphorus intake is an exceedingly important helpful measure. In their 3 cases, low calcium, low phosphorus diets with added calcium in the form of lactate was adequate therapy to keep the patients clinically comfortable and in positive balance so far as these 2 mineral elements were concerned.—J. E. H.

ANSPACH, W. E., AND W. M. CLIFTON. Hyperparathyroidism in children: report of two cases. *Am. J. Dis. Child.* 58: 540. 1939.

Hyperparathyroidism seen in a girl, 9 years old at onset of symptoms, exemplified the characteristic changes seen in older children and in adults. Removal of a parathyroid adenoma relieved the condition, with no recurrence during the subsequent 3 years. In the youngest patient thus far

reported symptoms appeared at 3 months and progressed for 2 years. Skeletal deformation and muscular weakness were most severe. Blindness, attributed to increased intracranial pressure secondary to distortion and fracture of a soft skull, developed during the 2nd year. Because of hypercalcemia and hypophosphatemia, together with initial normal renal function, this was believed to be a case of primary hyperparathyroidism, despite skeletal changes somewhat resembling those of renal rickets. A calcification as well as decalcification, as indicated by persistent loss of density of the endochondral zones, may be a feature of primary hyperparathyroidism during active body growth. The serum phosphorus value returned to normal during the pre-treatment observation period, with persistent hypercalcemia and increasing decalcification. Roentgen irradiation of the neck was followed by recalcification of bone and relief of polyuria and hypotonia.—N. B. G.

OLIVER, W. A. Acute hyperparathyroidism. *Lancet* 237: 240. 1939.

Two cases of parathyroid chief cell adenomata associated with widespread deposition of Ca are described in 2 women aged 56 and 57 respectively. The prominent symptoms in both instances were repeated nausea, vomiting, anorexia, loss of weight, obstinate constipation, and terminally increasing drowsiness. Physically severe dehydration, irregular pyrexia, tachycardia, no cardiac enlargement, and low blood pressure were present. Laboratory findings showed a slight hypochromic anemia, evidence of renal damage, but no significant change in alkali reserve. The terminal course was the same, rapidly increasing stupor, and progressively falling blood pressure. Pathologically, chief cell adenoma, glomerular necrosis with a minimal deposition of Ca, destruction of cardiac muscle fibers with deposition of Ca was found. Further there occurred a deposition of Ca in the stomach, lungs, heart, spleen, and liver. Arterial lesions were present in the kidney, femoral and other large arteries. The author contends that the most suitable form of treatment is the early recognition of the disease, fluid electrolyte replacement, and the early removal of the tumor.—L. B. S.

GREENE, J. A., AND L. W. SWANSON. Hypoparathyroidism, use and action of dihydrotachysterol (A.T. 10). *J. Iowa M. Soc.* 29: 275. 1939.

Five additional cases of hypoparathyroidism in which dihydrotachysterol has been successfully employed are reported. The drug has cumulative action and larger doses are required to raise serum Ca than to maintain it. Acute toxic manifestations are similar to those of parathyroid hormone and chronic overdosage produces metastatic calcification. The drug acts by increasing urinary P excretion and by increasing the Ca absorption from the enteric tract. A low Ca and P diet supplemented by Ca salts other than the phosphotes orally increase effectiveness of the drug and reduce the required dose. Serum Ca should be measured frequently during initial treatment and sufficiently often thereafter to be certain that hypercalcemia does not occur. The treatment of mild cases will require only the addition of Ca salts to a general diet, whereas moderate cases require a low Ca and P diet supplemented by Ca salts and severe cases require the addition of dihydrotachysterol. Parathyroid hormone and intravenous Ca therapy are indicated in severe tetany. Dihydrotachysterol is valuable in the treatment of chronic hypoparathyroidism but it should be regarded as a therapeutic adjunct, not as the sole treatment.—*Authors' Abst.*

JACOX, H. W., J. M. KING AND F. R. BAILEY. Parathyroidism; effect of irradiation of neck on repair of bone lesions. *Am. J. Roentgenol.* 41: 970. 1939.

The literature regarding the effects of irradiation on the parathyroids is reviewed. A typical example of parathyroid dysfunction but without demonstrable parathyroid tumor associated with advanced osteitis fibrosa generalisata is reported. The value of irradiation of the neck on the repair of bone lesions and the improvement in blood chemistry and clinical symptoms in this patient are emphasized. A selective therapeutic test of irradiation is suggested whenever possible as an aid in localizing a lesion of the parathyroids before operation. The relative value of surgery or irradiation regarding permanency of results remains to be determined. The patient returns to the hospital because a renal calculus became lodged in the ureter producing pyelonephritis. She died in uremic coma. At autopsy extensive hepatic and renal damage was disclosed, but no parathyroid tumor could be found.—*Authors' Abst. (abbreviated).*

PINEAL

DAVIS, L., AND J. MARTIN. Results of experimental removal of pineal gland in young mammals. *Arch. Neurol. & Psychiat.* 43: 23. 1940.

The old problem of the effects of pinealectomy was restudied in young rats, cats and dogs. Eleven litters of rats of which 57 individuals survived the complete operation to autopsy were used; also 71 cats comprising 19 litters and 2 litters of dogs of 3 individuals each. Litter-mate controls were used throughout. The animals were allowed to reach maturity and frequent observations were made as to somatic and sexual growth and behavior. No behavior changes were noted in the pinealectomized rats. Sexual maturity was attained at the same

age in both control and lesion animals. There was some indication of increased somatic development in the males at puberty. There were no differences in somatic or sexual maturation in the female control and the lesion cats. Both experienced normal estrus, and both had normal pregnancies; but their offspring, sired by pinealectomized males, were weak, and of 24 kittens only 2 lived more than 48 hours. The pinealectomized mothers showed a lack of maternal instinct and lactated inadequately. The pinealectomized males matured sexually 4 to 5 months earlier than their controls and showed precocious somatic development, still being larger than their controls at the time of full maturity of the normal cats. The lesion males were less playful, more aggressive and more belligerent than their controls. Second generation cats, either as midterm or term fetuses or as cats several weeks of age, did not show any gross variations from the normal animal of the same age. Serial sections of the cat brains did not show degeneration anterior to the habenular nuclei. No significant difference was noted between the mature control and the lesion animals in the histologic picture of the thyroid, adrenals and ovaries. The testes and the hypophyses of the pinealectomized males were larger than those of the control males, and the histologic appearance of these organs reflected the gross state. One pinealectomized male dog outstripped his control both as to sexual development and body size before 10 months of age. No final conclusions have yet been drawn; but the work is being continued. The results to date indicate that the pineal has a glandular function; that it is probably influential in early life; and in some undetermined way has a regulatory relation with sexual and somatic development. —R. G. H.

THYROID

Arnold, L. E. An attempt to control fetal weight. *Am. J. Obst. & Gynec.* 39: 99. 1940.

U.S.P. thyroid, grains 3 to 6 daily, was used in the last 4 or more months of 116 pregnancies, without food limitations, and the 116 normal babies had an average birth weight of 6.8 lbs. The mothers had no signs of hyperthyroidism, preeclampsia, or eclampsia, and there was an average maternal weight gain of 18.2 lbs.—E. L. S.

Man, E. B., E. F. Gildea and J. P. Peters. Serum lipoids and proteins in hyperthyroidism. *J. Clin. Investigation* 19: 43. 1940.

Continuing earlier work on hypothyroidism the authors report that cholesterol, lipoids, fatty acids are low in hyperthyroidism, but that there are too many cases where the normal range and the low values overlap to make these determinations of diagnostic value in individual cases. The cholesterol values tend to rise after use of iodine or thyroidectomy. Cholesterol was as useful as any other determination on lipoids. Protein changes were slight. There was no direct correlation between basal rates and cholesterol levels.—E. L. S.

Muller, G. P. and J. M. Surver. Thyrotoxic reactions following major operations. *Pennsylvania M. J.* 43: 642. 1940.

This paper, illustrated by 4 case histories, deals with unrecognized hyperthyroidism which develops following operation for unrelated conditions. A severe major operation such as hysterectomy, gastric resection, cholecystectomy, etc., might precipitate a thyroid crisis in a thyrotoxic individual, and a number of such instances are reported. In the literature cases are reported in which tonsillectomy, extraction of teeth, cystoscopy, and the injection of varicose veins have been the precipitating causes. The authors believe that many of these crises must have occurred in individuals while suffering from moderate symptoms of undetected hyperthyroidism. It is possible to overlook the thyrotoxic state in a patient referred to them for some unrelated condition. The hyperthyroidism may be present in a mild degree and yet the surgical procedure may precipitate a hyperthyroidism which has not been recognized and which rapidly develops into crisis and even death. If a toxic goiter is recognized, no operation should be done prior to the thyroidectomy except those of great emergency.—I. B.

Savy, P., R. Froment and M. Jeune. Cardiovascular disturbances and myxedema (Troubles cardio-vasculaires et myxoedeme). *Ann. méd.* 45: 216. 1939.

Two types of cardiovascular disturbances are observed in myxedema. In the first group the myxedematous conditions are directly responsible for the vascular changes and the latter disappear when the myxedema subsides with endocrine medication. Such disturbances are diminution of the voltage in the E.K.G. tracing, a more or less developed hypertrophy of the heart, some hypertensive symptoms, and, more seldom, angina type symptoms. All these symptoms are of benign character and easily cured, if they are not of too long standing. The second group comprises cases of long standing. They usually are aggravated by thyroid medication. The prognosis is unfavorable; their relation to the myxedema is uncertain, or at least indirect. This kind includes cases of coronary disease with or without angina symptoms, with or without infarcts, of persistent hypertension of high degree and certain types of cardiac insufficiency.—A. E. M.

Daniels, L. P. On the significance of the various phenomena of the Basedow complex. *Acta med. Scandinav.* 98: 561. 1939.

A clinical condition belonging to the Basedow

complex can be produced by feeding thyroxin or thyroid tissue. The histological picture found in the thyroid in the Basedow complex shows accumulations of lymphoid cells but thyrotropic hormone causes hyperplaxia only without increases in lymphoid tissue. Thyrotropic hormone is increased in the blood of patients with hypothyroid states and decreased in cases of hyperthyroidism. Exophthalmos can be produced by injections of thyrotropic hormone but not by thyroxin. Severe cases show marked mental instability. The author believes that the disease is a complex involving the thyroid, hypophysis and diencephalon.—J. M. L.

GREENFIELD, J., AND G. M. CURTIS. Acute suppurative thyroiditis during childhood. *Am. J. Dis. Child.* 58: 837. 1939.

A case is reported in a 10-year old boy who showed a B.M.R. of −16% and an iodine content of 2.47% 2 months after incision and drainage of a suppurative thyroiditis. Treatment of 1 grain of desiccated thyroid substance once a day and 10 mg. of iodine once a week for 2½ years resulted in an increase in the blood iodine to 5.57 (which is slightly above normal by Matthews' new method), an acceleration in bone development and and growth, and improvement in general symptomatology.—M. B. G.

LANGMANN, A. G., AND H. BRUCH. Carcinoma of the thyroid gland in children—report of a case associated with multiple anomalies of development, with studies of basal metabolism, serum cholesterol and creatine excretion after thyroidectomy. *Am. J. Dis. Child.* 56: 616. 1938.

A case of carcinoma of the thyroid gland in a 4-year old girl, with successful removal, is reported. Twenty other cases under 15 years of age are analyzed as to type and method of treatment. Carcinoma of the thyroid in children usually is of a lower grade of malignancy than in adults and offers a better prognosis. Laboratory studies revealed that operation was followed by a drop in B.M.R., rise in blood cholesterol and a drop in urinary creatine. Treatment by desiccated thyroid substances was followed by a rise in B.M.R., drop in cholesterol and rise in creatine.—M. B. G.

TALBOT, N. B., J. WORCESTER AND A. STEWART. New creatinine standard for basal metabolism and its clinical application. *Am. J. Dis. Child.* 58: 506. 1939.

The authors studied creatinine excretion and basal energy metabolism in children of both sexes between 5 and 14 years of age. A creatinine standard for metabolism was formulated. They considered that the correlation between total basal caloric output and creatinine excretion is as accurate as that between caloric output and weight in normal children and perhaps more accurate in those who are abnormal. Thyroid therapy showed that creatinine excretion is independent of the rate of metabolism; in all cases but 1, thyroid substance caused definite elevation in total caloric output but did not influence creatinine excretion. —M. B. G.